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Synthesis and antiproliferative evaluation of 23-hydroxybetulinic acid derivatives

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ABSTRACT

Based on structural modifications of the natural 23-hydroxybetulinic acid, a series of novel its derivatives had been synthesized. The new compounds were screened for *in vitro* antiproliferative activity against cancer cell lines HeLa, MCF-7, HepG2, B16 and A375 using doxorubicin as a reference. The vast majority of derivatives had exhibited potent tumor growth inhibitory activity than original compound. The derivatives **4**, **5**, **7**, **20**, **23**, **26**, **43** and **44** with IC_{50} values lower than 10 μM on all tested cell lines were regarded as the most promising compounds. The structure–activity relationships of 23-hydroxybetulinic acid derivatives were also discussed in the present investigations.

1. Introduction

Lupane triterpenoids such as betulinic acid are prevalent in natural sources and have various biological activities. Previous experimental and epidemiological studies have indicated that betulinic acid as well as its analogues-betulin and 23-hydroxybetulinic acid (Fig. 1) may be developed as potent anti-HIV and anti-tumor drugs [1–6]. A great deal of investigations on the structural modifications of betulinic acid and betulin were carried out, and many derivatives with excellent anti-HIV and anti-tumor activities have been obtained [3,7–20]. The 3-*O*-(3′,3′-dimethylsuccinyl)-betulinic acid (DSB) [19,20], one of the most famous derivative of betulinic acid, is currently under Phase-IIb clinical evaluation [10]. Nevertheless, there is rare report about the structural modifications of 23-hydroxybetulinic acid.

The 23-hydroxybetulinic acid, isolated from the root of *Pulsatilla chinensis*, displayed similar anti-HIV and anti-tumor activities as betulinic acid [21–24]. Besides the structural similarity and pharmacological comparability of 23-hydroxybetulinic acid with betulinic acid and betulin, the glycogen phosphorylase (GP) inhibitory activity of 23-hydroxybetulinic acid has also been investigated. A series of 23-hydroxybetulinic acid derivatives exhibiting improved GP inhibitory potencies were reported by literature [21]. Furthermore, 23-hydroxybetulinic acid exerted a synergistic effect on the cytotoxicity of doxorubicin (DOX) *in vitro* and *in vivo* in the recent study [22]. The results indicated that 23-hydroxybetulinic acid had the prospect to be developed as a novel chemosensitizer. Meanwhile, many attentions have also been paid to the antiproliferative mechanism of 23-hydroxybetulinic acid. Previous pharmacological studies suggested that the antiproliferative effect of 23-hydroxybetulinic acid was mediated by apoptotic induction and down-regulation of pro-apoptotic gene *bcl*-2. The telomerase may play a critical role in 23-hydroxybetulinic acid induced apoptosis [23]. This apoptosis inducing ability along with the apparent lack of toxicity in normal cells has made 23-hydroxybetulinic acid as a promising and potential anti-tumor agent [22,23].

Modification of potential plant-derived natural products is continuing goals of our laboratory. Suitable modification at right positions of these natural products may yield analogues with significantly improved potency. The structure of 23-hydroxybetulinic acid consists of a 30-carbon skeleton which has four available sites for chemical modifications at C-3, C-23, C-17 and C-19. A set of 17-carboxylic acid modified 23-hydroxybetulinic acid ester derivatives which exhibited slightly improved antiproliferative potencies were reported previously [24]. By utilizing the structure–activity relationships obtained by the previous betulinic acid and betulin modification researches, we conducted

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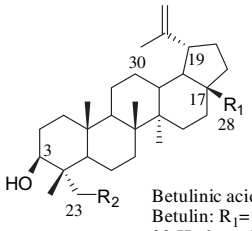


Betulinic acid: R_1= COOH R_2= H
Betulin: R_1= CH_2OH R_2= H
23-Hydroxy betulinic acid: R_1= COOH R_2= OH

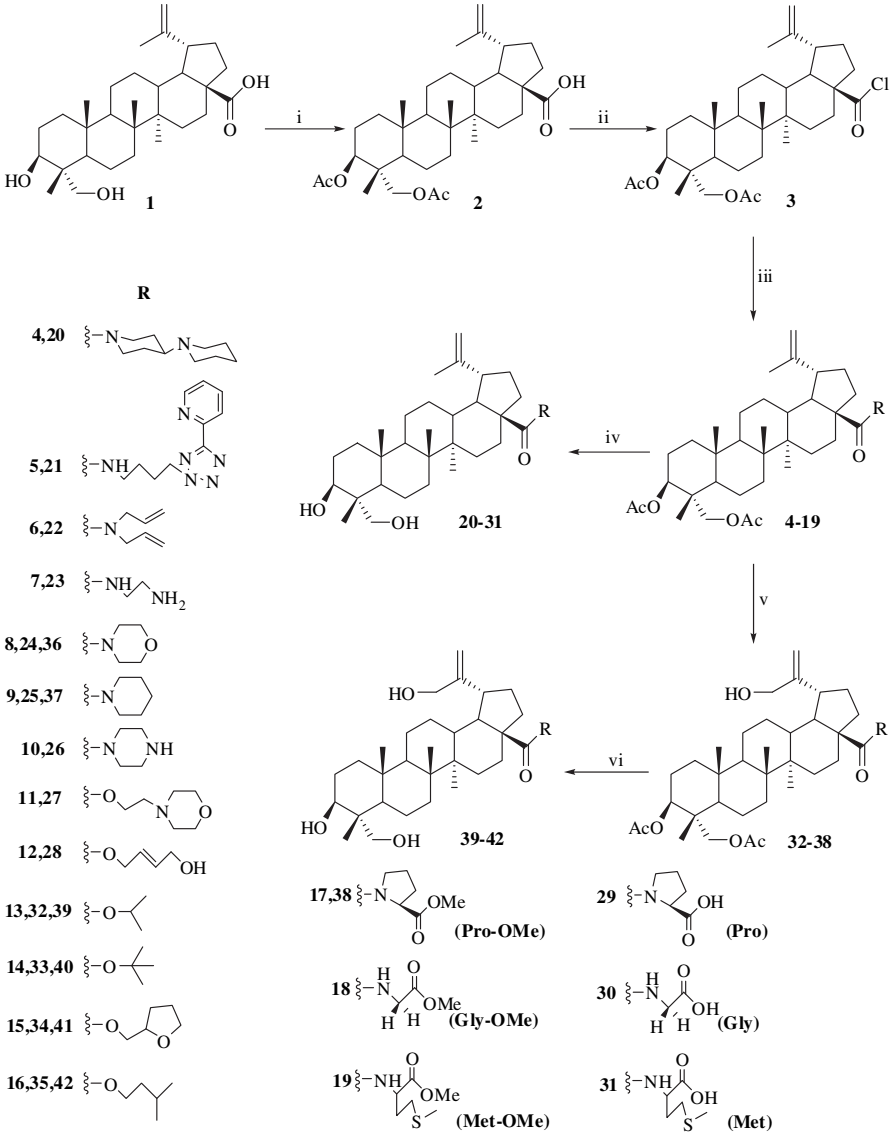
Fig. 1. The chemical structures of betulinic acid, betulin and 23-hydroxybetulinic acid.

the synthesis and antiproliferative activity evaluation of novel 23-hydroxybetulinic acid derivatives modified at C-3, C-23, C-17 and C-19 sites. Based on the observed antiproliferative data, preliminary structure–activity relationships have been established.

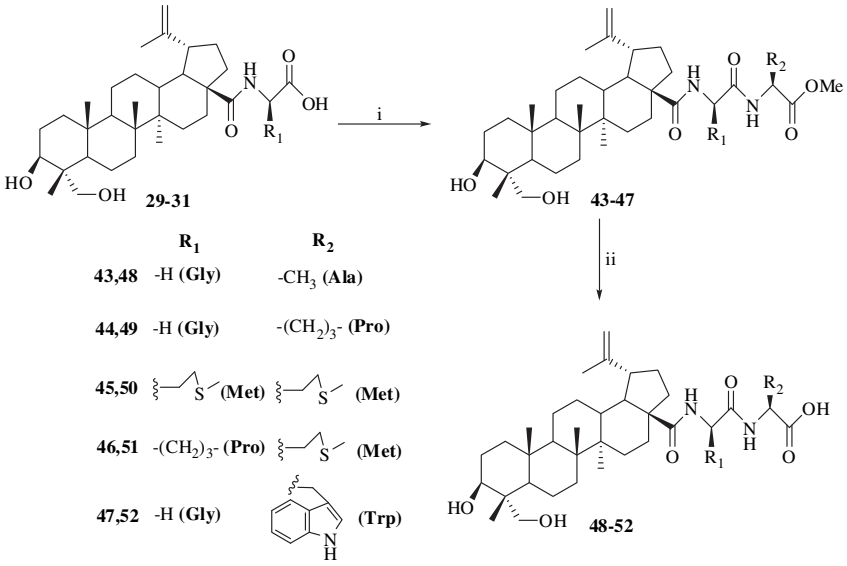
2. Results and discussion

2.1. Chemistry

Firstly, a series of 23-hydroxybetulinic derivatives bearing the C-17 modified carboxylate or amide have been designed and synthesized. Furthermore, based on the structural availabilities of these derivatives, a set of C-30 modified derivatives were also obtained. The general procedure for the synthesis of derivatives **4**–**42** was described in Scheme 1. The 23-hydroxybetulinic acid (**1**) was isolated by the laboratory of Prof. Wen-Cai Ye. It was treated with acetic anhydride in pyridine to yield 3,23-*O*-diacetyl betulinic acid (**2**). Compound **2** upon reaction with oxalyl chloride using DMF as a catalyst in CH_2Cl_2 afforded 3,23-*O*-diacetyl-17-acyl chloride (**3**). Upon reaction with 1,4′-dipiperidine, 4-(5-pyridine-2-tetrazolium-1-)-*n*-butylamine, diallylamine, ethylendiamine, morpholine, piperidine, piperazidine, 4-(2-hydroxyethyl) morpholine, *cis*-2-butene-1,4-diol, isopropyl alcohol, *tert*-butyl alcohol, tetrahydrofurfuryl alcohol, isopentyl



Scheme 1. Synthesis of derivatives **4**–**42**. Reagents and conditions: (i) Ac_2O/Pyr, rt, 8 h; (ii) $(COCl)_2/CH_2Cl_2$/cat.DMF, rt, 4 h; (iii) RNH, RNH_2 or ROH/CH_2Cl_2, rt or reflux, 8–72 h; (iv) $NaOH/THF/CH_3OH$, reflux, 4 h; (v) *m*-CPBA/$CHCl_3$, reflux, 6 h; (vi) $NaOH/THF/CH_3OH$, reflux, 4 h.



Scheme 2. Synthesis of derivatives **43**–**52**. Reagents and conditions: (i) EDC·HCl/HoBt/CH_2Cl_2, reflux, 8–12 h; (ii) NaOH/THF/CH_3OH, reflux, 4 h.

acohol, L-proline methyl ester hydrochloride, glycine methyl ester hydrochloride and L-methionine methyl ester hydrochloride, yielded corresponding 3,23-*O*-diacetyl-17-carboxylate or amide derivatives **4**–**19**. Compounds **4**–**12** as well as **17**–**19** were then hydrolyzed to give corresponding 3,23-dihydroxy-17-carboxylate or amide derivatives **20**–**28** and **29**–**31**. Meanwhile, compounds **13**–**17** and **8**–**9** were treated with metachloroperbenzoic acid (*m*-CPBA) to yield subsequent 3,23-*O*-diacetyl-30-hydroxy-17-carboxylate or amide derivatives **32**–**38**. Finally, compounds **32**–**35** were hydrolyzed to afford 3,23-dihydroxy-30-hydroxy-17-carboxylate derivatives **39**–**42**.

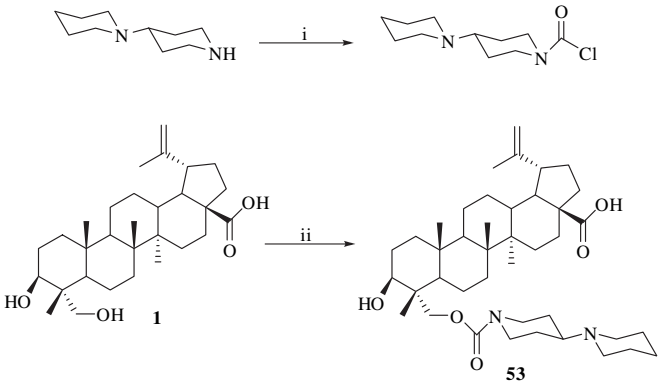
The previous researches have revealed that amino acid conjugates of betulinic acid have improved water solubility and were selectively antiproliferative toward cancer cells [12]. Thus, we have designed and synthesized a series of 23-hydroxybetulinic acid derivatives with conjugation of amino acids and dipeptides. The general procedures for the synthesis of the amino acid conjugates derivatives **29**–**31** and the dipeptide conjugates compounds **43**–**52** were described in Scheme 1 and Scheme 2. The amino acid conjugates derivatives **29**–**31** upon reaction with L-alanine methyl ester hydrochloride, L-proline methyl ester hydrochloride, L-methionin methyl ester hydrochloride and L-tryptophane methyl ester hydrochloride in CH_2Cl_2 yielded corresponding 3,23-dihydroxy-17-dipeptide methyl ester derivatives **43**–**47**. Finally, compounds **43**–**47** were hydrolyzed to give corresponding 3,23-dihydroxy-17-dipiptide derivatives **48**–**52**.

The 23-hydroxybetulinic acid possessed a peculiar hydroxyl group at C-23 position, while the betulinic acid and betulin have not such a moiety. It is the first time to report a chemical modification at this hydroxyl group. We have introduced a 1,4′-dipiperidine-1-carbonyl moiety into this C-23 position by following the successful preparation of irinotecan. 1,4′-dipiperidine upon reaction with triposgene in dry CH_2Cl_2 afforded 1,4′-dipiperidine-1-carbonyl chloride, then it was reacted with 23-hydroxybetulinic acid to give 3-hydroxy-23-*O*-(1,4′-bipiperidine-1-carbonyl)betulinic acid (**53**) in a high yield (Scheme 3). Meanwhile, since compound **53** displayed excellent potency, we have also designed a set of 3-hydroxy-23-*O*-(1,4′-bipiperidine-1-carbonyl)-17-carboxylate or amide derivatives, unfortunately, none of the designed molecules was obtained. For instance, the compound **20** upon reaction with 1,4′-dipiperidine-1-carbonyl chloride in pyridine yielded derivative **54**, not the designed one (Scheme 4). It can be inferred that the C-17 region was close to the C-23 position in space, therefore, once the C-17 was occupied, the 1,4′-dipiperidine-1-carbonyl chloride was unable to attack the C-23 hydroxyl group due to the steric hindrance. Meanwhile, the excess triphosgene (used to prepare the 1,4′-dipiperidine-1-carbonyl chloride) in the reaction mixture was activated by nucleophile (pyridine or triethylamine) and then upon reaction with the C-23 hydroxyl group to afford 3-hydroxy-23-*O*-carbonochloridate-17-1,4′-bipiperidinyl betulinic amide and finally formed the compound **54**. The procedure of this kind of reaction was illustrated in Fig. 2.

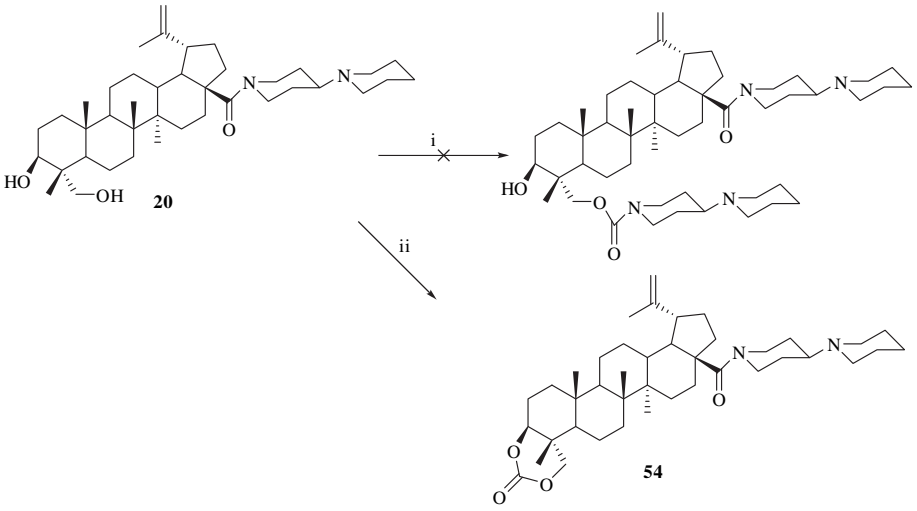
To investigate whether the carboxyl group at the terminal of the C-17 side chain is essential for the antiproliferative activities, compound **7** was selected for further modification. We introduced a 3-carboxypropanoyl in the terminal of the C-28 side chain of compound **7** by treating it with succinic anhydride. (Scheme 5)

2.2. Antiproliferative activity in vitro

To evaluate the anticancer potencies of these newly synthesized 23-hydroxybetulinic acid derivatives, the antiproliferative activities of compounds **1**–**55** were tested against five cancer cell lines,



Scheme 3. Synthesis of compound **53**. Reagents and conditions: (i) Triphosgene/Et_3N/Pyr, rt, 8 h; (ii) 1,4′-dipiperidine-1-carbonyl chloride/Pyr, rt, 16 h.



Scheme 4. Synthesis of derivative **54**. Reagents and conditions: (i) 1,4′-dipiperidine-1-carbonyl chloride/Pyr, rt; (ii) Triphosgene/Et_3N/Pyr, rt.

including HepG2 human hepatocellular carcinoma, HeLa human cervical adenocarcinoma, MCF-7 breast adenocarcinoma, B16 mice melanoma and A375 human melanoma cells by performing MTT assay. Doxorubicin was selected as positive control. These results were summarized in Table 1 and presented as the concentration of drug inhibiting 50% cell growth (IC_{50}).

2.3. Structure–activity relationship

The present results demonstrate that nearly all synthesized 23-hydroxybetulinic acid derivatives can markedly inhibit the proliferation of cancer cells. Among them, compounds **4**, **5**, **7**, **20**, **23**, **26**, **43** and **44** with an IC_{50} around 10 μM on all tested cell lines were the most promising derivatives. Comparing the activities of all derivatives with that of 23-hydroxybetulinic acid in each panel, it can be found that the vast majority of derivatives have shown a better antiproliferative profile than 23-hydroxybetulinic acid except **9**, **29**, **41**, **49**–**52** and **55**.

The results also revealed that, 3,23-*O*-diacetyl derivatives (e.g. **4**–**8**, **11**–**12**, **17** and **32**–**35**) were more potent than corresponding 3,23-dihydroxy compounds (e.g. **20**–**24**, **27**–**29** and **39**–**42**) in all tested cancer cell lines. This observation indicated that the acetyl at C-3 and C-23 positions markedly influenced the antiproliferative potential, which was in agreement with the previous betulinic acid researches [7–12]. Compared compound **9** with **10**, as well as **25** with **26**, it can be easily found that an electron-donating and hydrophilic substituent such as groups bearing an N atom at the terminal of C-17 side chain would benefit the potency. This also contributed to explain why compounds **4**–**5**, **7**, **23** as well as **26** bearing one or several N atoms at C-17 side chain, were the most potential derivatives. The C-17 ester derivatives (**11**, **12**) displayed improved activities than 23-hydroxybetulinic acid and were found to be as potent as C-17 amide compounds (**4**–**8**). The compounds **43**–**47** with C-17 dipeptide methyl ester exhibited extremely better potencies compared to their corresponding C-17 dipeptides derivatives **48**–**52**, suggesting that the carboxyl group at the terminal of the C-17 side chain was not essential for antiproliferative activity. To validate this observation, one of the most potential derivative **7** was selected for further investigation, compared compound **7** with **55**, it was also found that the 3′-*N*-succinic acid-4′-carbonyl derivative **55** displayed significantly decreased potency. This observation varied from some previous betulinic acid researches

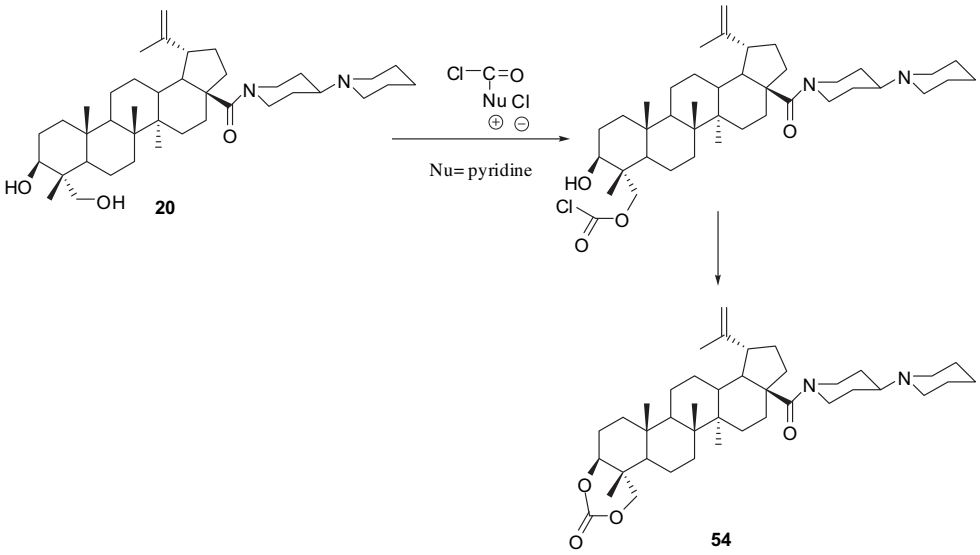
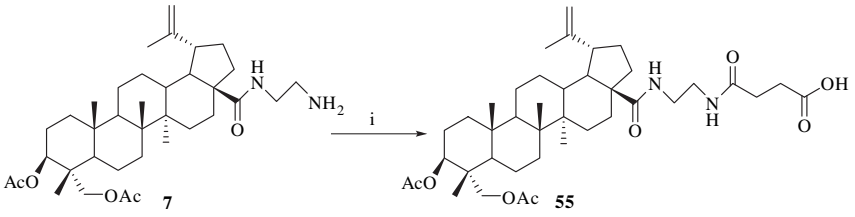


Fig. 2. The reaction mechanism for the synthesis of compound **54**.



Scheme 5. Synthesis of derivative **55**. Reagent and condition: (i) Succinic anhydride/DMAP/CH_2Cl_2, reflux, 10 h.

[12,25] suggesting a carboxyl group at this site would be favorable. The introduction of the 1′,4′-dipiperidine-1′-carbonyl at C-23 position yielded potent derivative **53** revealing that bulky, hydrophilic groups at C-23 position would be favored. Since the betulinic acid and betulin have not such a hydroxyl group at C-23 position, the hydroxyl group at C-23 site of 23-hydroxybetulinic acid is worthy of further modification and research. Compared 3,23-dihydroxy derivative **20** with corresponding 3,23-dioxylcarbonyl compound **54**, it was observed that protection of the C-3 and C-23 with dioxylcarbonyl group may result in decreased activities. The introduction of the hydroxyl at C-30 position yielded derivatives with slightly decreased antiproliferative activities (**36** and **38**) compared to C-30 unsubstituted compounds (**8** and **17**). Meanwhile, compared to those 3,23-diacetic-30-hydroxy derivatives, the 3,23-dihydroxy-30-hydroxy compounds (**39**–**42**) exhibited decreased antiproliferative potencies, indicating that a hydrophilic group at C-30 position may be unfavorable. In fact, most of the efforts on introducing various moieties into the C-19 site failed in yielding potential betulinic acid and betulin derivatives [5,26].

3. Conclusion

The chemically synthesized anticancer drugs in clinic usually displayed unacceptable adverse effects and toxicity. Nevertheless, the naturally occurring 23-hydroxybetulinic acid selectively killed cancer cells and was non-toxic to normal tissue in animal models. A large number of 23-hydroxybetulinic acid derivatives as possible anti-tumor agents were reported. The present study demonstrate that introduction with proper substituents at C-3, C-23 and C-17 positions of 23-hydroxybetulinic acid can produce various potentially important derivatives with improved antiproliferative activity. Derivatives **4**, **5**, **7**, **20**, **23**, **26**, **43** and **44** are potential lead compounds for the development of novel anti-tumor agent. Based on the observed antiproliferative evaluation, the structure–activity relationship of these derivatives has been established. In detail, the acetyl groups at both C-3 and C-23 positions would be favorable; a bulky, electron-donating and hydrophilic moiety at C-23 site may benefit the potency; a carboxyl group at the terminal of the C-17 side chain was not essential for the antiproliferative activity; the C-19 position was not eligible for a successful modification; modification at the C-17 site with suitable carboxylate or amide can produce potent derivatives. The results taken from present study can be served as a valuable guideline for further modification of 23-hydroxybetulinic acid with improved biological response.

4. Experimental

4.1. Chemistry

4.1.1. General

All reagents and solvents were purchased from commercial sources. Further purification and drying by standard methods were employed when necessary. For thin layer chromatography (TLC) analysis Qingdao haiyang GF_{254} silica gel plates were used. All NMR spectra were recorded on a Mercury-400 spectrometer in DMSO-d_6, $CDCl_3$, CD_3OD or pyridine-d_5. The chemical shift was reported in ppm using TMS as the internal standard. The coupling constant (J) was presented in hertz (Hz). Electrospray ionization mass spectra (ESI-MS) were obtained on a Finnigan LCQ Advantage MAX mass spectrometer (Applied Biosystems, 4000 Q TRAP). High-resolution mass spectra (HR-MS) were obtained on an Aglient 6210 series LC/MSD TOF mass spectrometer.

4.1.2. 3,23-O-Diacetyl betulinic acid (**2**)

To a solution of compound **1** (0.30 g, 0.63 mmol) in dry pyridine (12 mL) was added acetic anhydride (1 mL, 9.8 mmol), the solution was stirred at room temperature for 8 h. After reaction completion, 25 mL of ethyl acetate was added. And then the pH of solution was adjusted to 4–5 by 10% HCl. Then the organic layer was washed by brine (50 mL×3), dried with anhydrous Na_2SO_4 for 6 h. Filtered, the filtrate was concentrated, the residue was dissolved in dichloromethane and purified on a silica gel column (200 g, petroleum ether–ethyl acetate 10:1) to give **2** (0.32 g, 91.4%) as white foam. MS (ESI): m/z 579.5 $[M + Na]^+$; HR-ESI-MS: m/z 579.3652 $[M + Na]^+$ (calcd: 579.3656); ^1H NMR ($CDCl_3$, 400 MHz) δ: 0.80, 0.88, 0.93, 0.97 (each 3H, s, Me-24, -25, -26, -27), 1.69 (3H, s, Me-30), 1.79 (1H, m, H-18), 1.95 (1H, m, H-19), 2.01 (3H, s, 3-OAc), 2.06 (3H, s, 23-OAc), 3.02 (1H, m, H-3), 3.68 (1H, d, $J = 11.6$ Hz, H-23α), 3.83 (1H, d, $J = 11.6$ Hz, H-23β), 4.61 (1H, s, H-29α), 4.77 (1H, s, H-29β); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 12.8, 14.5, 16.0, 16.5, 17.9, 19.3, 20.9, 21.1, 23.1, 29.6, 36.9, 38.0, 38.3, 40.6, 40.6, 42.3, 46.9, 48.0, 49.2, 50.5, 56.3, 65.4, 74.5, 109.7, 150.3, 170.6, 171.0, 181.5.

4.1.3. 3,23-O-Diacetyl-17-acyl chloride (3)

To a solution of compound **2** (0.5 g, 0.87 mmol) in dry dichloromethane (30 mL) was added oxalyl chloride (0.5 mL, 2.65 mmol) and dry DMF (0.1 mL). The solution was stirred at room temperature for 4 h. After quenching the reaction, the solution was concentrated to give compound **3** (about 0.5 g) as yellow foam.

4.1.4. 3,23-O-Diacetyl-17-1,4′-bipiperidinyl betulinic amide (**4**)

To a solution of compound **3** (0.38 g, 0.66 mmol) in dry dichloromethane (40 mL) was added 1,4′-dipiperidine (0.66 g, 4.0 mmol). The solution was stirred and refluxed for 10 h. At which time the reaction was completed, the solution was concentrated, the residue was dissolved in ethyl acetate (40 mL) and 10% HCl (40 mL), the organic layer was separated and washed by brine (50 mL), dried with anhydrous Na_2SO_4. Filtered, the filtrate was concentrated, the residue was dissolved in methanol and purified on a silica gel column (200 g, ethyl acetate-methanol 3:1) to give **4** (0.40 g, 85.0%) as white foam. MS (ESI): m/z 708.6 $[M + H]^+$; ^1H NMR (CD_3OD, 400 MHz) δ: 0.84, 0.94, 0.97, 1.02 (each 3H, s, Me-24, -25, -26, and -27), 1.70 (3H, s, Me-30), 2.02 (1H, m, H-18), 1.95 (1H, m, H-19), 2.01 (3H, s, 3-OAc), 2.04 (3H, s, 23-OAc), 2.18

Table 1
Antiproliferative activities of compounds **1**–**55** against HeLa, MCF-7, HepG2, B16 and A375 cells.

Compounds	IC_{50} [a](μM ± SD)				
	HeLa	MCF-7	HepG2	B16	A375
1	46.22 ± 1.34	46.61 ± 1.69	41.41 ± 2.94	18.68 ± 1.24	41.99 ± 4.86
4	10.80 ± 1.06	6.86 ± 0.73	11.14 ± 0.68	9.57 ± 0.85	8.42 ± 0.97
5	17.87 ± 0.28	16.10 ± 1.10	3.91 ± 0.57	4.75 ± 0.29	1.81 ± 0.43
6	9.84 ± 0.32	47.10 ± 1.07	8.41 ± 0.71	>100	19.04 ± 0.89
7	7.39 ± 0.56	3.93 ± 0.60	7.99 ± 0.77	4.61 ± 0.32	9.99 ± 0.27
8	7.47 ± 0.65	26.44 ± 0.88	9.74 ± 0.79	18.48 ± 1.64	4.33 ± 0.35
9	69.20 ± 6.47	>100	66.34 ± 6.04	>100	>100
10	18.84 ± 1.61	4.89 ± 0.63	17.65 ± 0.72	13.07 ± 1.23	18.79 ± 1.69
11	9.18 ± 0.28	97.57 ± 8.75	12.28 ± 0.42	24.21 ± 2.69	8.71 ± 0.93
12	16.35 ± 1.67	15.91 ± 0.17	7.43 ± 0.80	>100	5.72 ± 0.76
13	nt[b]	nt	nt	nt	nt
14	nt	nt	nt	nt	nt
15	nt	nt	nt	nt	nt
16	nt	nt	nt	nt	nt
17	9.78 ± 0.18	27.22 ± 2.15	9.78 ± 0.97	93.67 ± 6.70	5.83 ± 0.53
18	nt	nt	nt	nt	nt
19	nt	nt	nt	nt	nt
20	8.42 ± 0.45	6.78 ± 0.85	12.64 ± 1.50	5.71 ± 0.26	9.67 ± 0.67
21	46.26 ± 3.65	17.68 ± 0.97	48.27 ± 4.83	66.91 ± 3.46	30.58 ± 1.02
22	59.01 ± 5.57	17.70 ± 0.54	59.77 ± 5.44	60.40 ± 6.44	17.18 ± 0.52
23	8.92 ± 0.74	2.93 ± 0.32	9.57 ± 0.12	8.38 ± 0.81	4.83 ± 0.26
24	34.29 ± 1.16	19.84 ± 1.51	41.97 ± 1.45	42.70 ± 3.03	25.06 ± 2.64
25	66.45 ± 2.97	16.63 ± 1.16	68.60 ± 3.82	43.66 ± 2.02	17.88 ± 0.80
26	7.12 ± 1.27	2.27 ± 0.35	8.85 ± 0.42	4.39 ± 0.46	2.30 ± 0.19
27	>100	34.30 ± 0.67	59.21 ± 5.29	45.85 ± 3.34	34.24 ± 3.93
28	44.44 ± 4.12	36.03 ± 0.80	38.00 ± 2.98	19.08 ± 1.64	55.69 ± 4.74
29	95.61 ± 3.79	>100	72.21 ± 5.64	87.34 ± 3.44	>100
30	nt	nt	nt	nt	nt
31	nt	nt	nt	nt	nt
32	19.46 ± 1.39	17.21 ± 0.96	10.71 ± 1.34	23.65 ± 1.63	21.54 ± 1.54
33	23.28 ± 1.84	14.12 ± 1.92	15.75 ± 1.62	49.45 ± 1.29	34.72 ± 2.75
34	17.79 ± 1.72	33.65 ± 0.87	18.65 ± 2.54	41.80 ± 3.89	12.47 ± 1.54
35	20.84 ± 1.21	15.10 ± 1.49	10.72 ± 0.66	35.88 ± 1.27	7.80 ± 0.82
36	18.31 ± 1.23	17.32 ± 0.55	20.68 ± 1.63	39.27 ± 3.94	31.84 ± 1.17
37	11.02 ± 1.27	22.20 ± 2.10	11.27 ± 1.11	64.26 ± 5.52	53.74 ± 4.57
38	13.08 ± 1.01	31.98 ± 1.37	10.00 ± 1.18	68.97 ± 2.73	11.75 ± 1.56
39	22.27 ± 1.46	16.57 ± 1.22	20.98 ± 1.80	38.57 ± 0.99	21.20 ± 2.16
40	30.65 ± 0.82	16.40 ± 0.62	22.68 ± 1.64	84.08 ± 8.31	42.71 ± 3.58
41	79.35 ± 5.95	76.30 ± 8.44	81.23 ± 6.97	64.35 ± 0.35	70.37 ± 5.13
42	20.85 ± 1.40	12.53 ± 1.76	34.62 ± 1.87	51.20 ± 4.30	17.94 ± 1.29
43	8.27 ± 0.91	8.95 ± 0.20	12.09 ± 1.01	11.31 ± 1.14	5.01 ± 0.60
44	4.84 ± 0.51	7.58 ± 0.62	9.33 ± 0.69	8.23 ± 0.87	3.34 ± 0.37
45	44.39 ± 3.95	66.09 ± 4.30	23.05 ± 1.73	20.39 ± 1.89	26.80 ± 0.71
46	41.26 ± 3.19	19.77 ± 1.56	40.46 ± 2.27	20.73 ± 0.44	30.14 ± 2.60
47	66.33 ± 6.64	>100	46.79 ± 4.34	44.35 ± 2.75	>100
48	22.10 ± 1.74	17.25 ± 1.31	32.36 ± 2.20	22.25 ± 0.82	18.38 ± 0.41
49	>100	>100	>100	>100	>100
50	>100	84.72 ± 6.72	>100	65.09 ± 3.47	89.30 ± 0.98
51	81.13 ± 8.31	41.30 ± 1.51	91.70 ± 6.82	76.46 ± 0.31	66.50 ± 5.28
52	>100	>100	>100	>100	>100
53	12.52 ± 1.85	9.95 ± 0.98	18.54 ± 1.36	7.25 ± 1.25	9.23 ± 0.67
54	20.55 ± 2.06	17.56 ± 0.52	10.28 ± 0.96	8.69 ± 1.53	20.41 ± 1.06
55	72.90 ± 6.68	51.00 ± 2.14	76.09 ± 3.57	82.57 ± 3.37	59.53 ± 4.83
DOX	0.13 ± 0.02	0.19 ± 0.02	0.19 ± 0.02	0.14 ± 0.02	0.13 ± 0.01

Antiproliferative activity was determined by MTT assay as shown in Experimental 4.2 part. All data are presented as means ± standard deviation of at least three independent experiments.
DOX: Doxorubicin.
[a] IC_{50}: concentration of the tested compound that inhibits 50% of cell growth.
[b] nt: not test.

(4H, m, H-2″, 6″), 2.90 (1H, m, H-3), 2.95 (4H, m, H-2′, 6′), 2.91 (1H, d, J = 11.8 Hz, H-23α), 3.81 (1H, d, J = 11.8 Hz, H-23β), 4.59 (1H, s, H-29α), 4.72 (1H, s, H-29β); ^{13}C NMR (CD_3OD, 100 MHz) δ: 18.0, 18.6, 20.3, 21.1, 22.1, 22.5, 23.7, 24.2, 25.4, 25.7, 28.2, 29.3, 29.6, 32.4, 33.7, 43.7, 36.5, 38.3, 39.5, 39.7, 40.6, 43.2, 43.3, 44.4, 48.6, 49.7, 50.8, 51.0, 52.6, 53.6, 55.2, 57.4, 66.1, 67.5, 77.2, 80.9, 111.3, 153.8, 173.9, 173.9, 176.9.

The compounds **5**–**10** were obtained by using the similar synthetic procedure of compound **4**.

*4.1.5. 3,23-O-Diacetyl-17-[4-(5-(pyridin-2-yl)-2H-tetrazol-2-yl)-n-butyl]betulinic amide (**5**)*

White foam, yield: 87.0%. MS (ESI): m/z 758.3 $[M + H]^+$, 780.1 $[M + Na]^+$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.62, 0.84, 0.88, 1.27 (each 3H, s, Me-24, -25, -26, and -27), 1.43 (3H, s, Me-30), 1.78 (1H, m, H-18), 2.14 (1H, m, H-19), 2.26 (6H, s, 3,23-OAc), 3.00 (1H, m, H-3), 3.62 (1H, m, H-2′α), 3.64 (1H, m, H-2′β), 4.07 (1H, m, H-5′α), 4.54 (1H, d, J = 8.0 Hz, H-23α), 4.59 (1H, d, J = 8.0 Hz, H-23β), 5.02 (1H, m, H-5′β), 5.29 (1H, s, H-29α), 5.50 (1H, s, H-29β), 7.42 (1H, m, H-14′), 7.54 (1H,

m, H-15′), 8.01 (2H, m, H-16′, 13′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ:10.2, 13.7, 14.7, 15.7, 19.8, 20.7, 21.0, 22.5, 32.4, 33.1, 34.1, 35.1, 40.7, 45.8, 49.9, 51.1, 52.0, 63.5, 69.3, 70.4, 71.9, 73.2, 75.5, 79.7, 82.5, 101.4, 128.3, 129.6, 129.7, 130.4, 132.3, 132.8, 138.4, 163.2, 165.1, 169.5.

*4.1.6. 3, 23-O-Diacetyl-17-diallyl betulinic amide (**6**)*

White foam, yield: 91.0%. MS (ESI): *m/z* 536.4 $[M + H]^+$, 658.5 $[M + Na]^+$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.80, 0.88, 0.94, 0.95 (each 3H, s, Me-24, -25, -26, and -27), 1.68 (3H, s, Me-30), 1.83 (1H, m, H-18), 1.96 (1H, m, H-19), 2.01 (3H, s, 3-OAc), 2.06 (3H, s, 23-OAc), 3.04 (1H, m, H-3), 3.67 (1H, d, J = 11.6 Hz, H-23α), 3.86 (1H, d, J = 11.6 Hz, H-23β), 3.87 (2H, m, H-2′), 4.09 (2H, m, H-5′), 4.58 (1H, s, H-29α), 4.76 (1H, s, H-29β), 5.15 (4H, m, H-4′, 7′), 5.74 (2H, m, H-3′, 6′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 12.8, 14.6, 16.0, 16.6, 17.9, 20.9, 21.2, 23.1, 25.6, 29.8, 31.4, 32.2, 34.0, 36.8, 37.0, 40.6, 40.7, 41.9, 45.6, 48.1, 50.9, 52.9, 54.8, 65.4, 74.5, 109.0, 133.6, 151.4, 170.6, 171.0, 174.4.

*4.1.7. 3,23-O-Diacetyl-17-(2-aminoethyl)betulinic amide (**7**)*

White foam, yield: 76.9%. MS (ESI): *m/z* 599.8 $[M + H]^+$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.74, 0.83, 0.87, 0.97 (each 3H, s, Me-24, -25, -26, and -27), 1.68 (3H, s, Me-30), 1.97 (1H, m, H-18), 2.01 (3H, s, 3-OAc), 2.06 (3H, s, 23-OAc), 2.39 (1H, m, H-19), 2.44 (2H, m, H-3′), 3.06 (1H, m, H-3), 3.24 (2H, t, H-2′), 3.68 (1H, d, J = 11.5 Hz, H-23α), 3.84 (1H, d, J = 11.5 Hz, H-23β), 4.60 (1H, s, H-29α), 4.75 (1H, s, H-29β), 5.39 (1H, s, H-1′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 12.8, 13.0, 14.5, 15.8, 16.1, 16.5, 17.9, 19.3, 20.8, 21.1, 23.1, 23.5, 30.6, 36.9, 38.0, 39.3, 40.5, 40.5, 40.7, 42.4, 46.8, 48.0, 50.6, 55.6, 65.4, 74.4, 109.3, 150.6, 170.6, 170.9, 171.0.

*4.1.8. 3,23-O-Diacetyl-17-morpholinyl betulinic amide (**8**)*

White foam, yield: 83.3%. MS (ESI): *m/z* 627.0 $[M + H]^+$; 649.0 $[M + Na]^+$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.82, 0.89, 0.95, 0.97 (each 3H, s, Me-24, -25, -26, and -27), 1.69 (3H, s, Me-30), 1.95 (1H, m, H-18), 2.02 (3H, s, 3-OAc), 2.07 (3H, s, 23-OAc), 2.10 (1H, m, H-19), 2.99 (1H, m, H-3), 3.66 (8H, m, H-2′, 3′, 5′, 6′), 3.70 (1H, d, J = 11.6 Hz, H-23α), 3.85 (1H, d, J = 11.6 Hz, H-23β), 4.60 (1H, s, H-29α), 4.74 (1H, s, H-29β); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 12.7, 14.4, 15.9, 16.5, 17.8, 20.7, 21.0, 29.6, 31.1, 33.9, 36.7, 36.9, 37.9, 40.4, 40.5, 41.7, 45.5, 48.0, 50.7, 52.5, 54.3, 66.8, 74.4, 109.1, 151.0, 170.5, 170.8, 173.5.

*4.1.9. 3,23-O-Diacetyl-28-piperidinyl betulinic amide (**9**)*

White foam, yield: 74.0%. MS (ESI): *m/z* 646.6 $[M + Na]^+$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.85, 0.94, 0.95, 0.99 (each 3H, s, Me-24, -25, -26, and -27), 1.70 (3H, s, Me-30), 1.95 (1H, m, H-18), 2.01 (3H, s, 3-OAc), 2.06 (3H, s, 23-OAc), 2.10 (1H, m, H-19), 3.04 (1H, m, H-3), 3.48 (4H, m, H-2′, 6′), 3.67 (1H, d, J = 11.5 Hz, H-23α), 3.83 (1H, d, J = 11.5 Hz, H-23β), 4.57 (1H, s, H-29α), 4.76 (1H, s, H-29β); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 12.8, 14.5, 16.0, 16.6, 20.9, 21.1, 21.2, 23.1, 25.5, 26.1, 29.8, 31.3, 32.4, 36.8, 36.9, 37.9, 40.5, 40.6, 41.8, 45.7, 48.0, 50.8, 52.7, 54.6, 65.4, 74.5, 109.0, 151.5, 170.6, 171.0, 173.1.

*4.1.10. 3,23-O-Diacetyl-28-piperizinyl betulinic amide (**10**)*

White foam, yield: 74.1%. MS (ESI): *m/z* 625.8 $[M + H]^+$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.81, 0.88, 0.94, 0.95 (each 3H, s, Me-24, -25, -26, and -27), 1.68 (3H, s, Me-30), 1.95 (1H, m, H-18), 2.01 (3H, s, 3-OAc), 2.06 (3H, s, 23-OAc), 2.12 (1H, m, H-19), 2.48 (4H, m, H-3′, 5′), 2.89 (1H, m, H-3), 3.60 (4H, m, H-2′, 6′), 3.68 (1H, d, J = 11.6 Hz, H-23α), 3.84 (1H, d, J = 11.6 Hz, H-23β), 4.58 (1H, s, H-29α), 4.72 (1H, s, H-29β); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 12.8, 14.5, 16.1, 16.6, 17.9, 19.6, 20.9, 21.2, 23.1, 29.7, 31.3, 34.0, 36.9, 37.0, 38.0, 40.6, 40.7, 41.8, 45.7, 48.1, 50.9, 52.7, 54.5, 65.5, 74.5, 109.1, 151.3, 170.6, 170.9, 173.5.

*4.1.11. 3,23-O-Diacetyl-17-(2-morpholinylethyl)betulinic ester (**11**)*

To a solution of compound **3** (0.30 g, 0.52 mmol) in dry dichloromethane (30 mL) was added 4-(2-hydroxyethyl) morpholine (0.1 mL, 5.22 mmol). The solution was stirred at room temperature for 8 h. At which time the reaction was completed, the solution was washed by water (40 mL) and brine (40 mL), dried with anhydrous Na_2SO_4. Filtered, the filtrate was concentrated, the residue was dissolved in dichloromethane and purified on a silica gel column (200 g, petroleum ether–ethyl acetate 1:1) to give **11** (0.18 g, 51.4%) as white foam. MS (ESI): *m/z* 670.8 $[M + H]^+$; 692.9 $[M + Na]^+$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.80, 0.87, 0.92, 0.96 (each 3H, s, Me-24, -25, -26, and -27), 1.68 (3H, s, Me-30), 1.90 (1H, m, H-18), 1.98 (1H, m, H-19), 2.01 (3H, s, 3-OAc), 2.06 (3H, s, 23-OAc), 2.51 (4H, m, H-4′, 8′), 2.67 (2H, t, H-2′), 3.01 (1H, m, H-3), 3.70 (4H, m, H-5′, 7′), 3.72 (1H, d, J = 11.6 Hz, H-23α), 3.83 (1H, d, J = 11.6 Hz, H-23β), 4.21 (2H, t, H-1′), 4.60 (1H, s, H-29α), 4.74 (1H, s, H-29β); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 12.8, 14.5, 16.0, 16.5, 17.8, 19.2, 20.9, 21.2, 29.5, 36.9, 36.9, 38.1, 40.5, 40.6, 42.3, 46.9, 47.9, 49.2, 50.5, 53.7, 56.4, 57.2, 60.7, 65.3, 66.9, 74.4, 109.6, 150.4, 170.6, 171.0, 175.8.

Compounds **12**–**16** were obtained by using the similar synthetic procedure of compound **11**.

*4.1.12. 3,23-O-Diacetyl-17-(E-4-hydroxybut-2-enyl)betulinic ester (**12**)*

White foam, yield: 71.9%. MS (ESI): *m/z* 649.6 $[M + Na]^+$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.81, 0.88, 0.91, 0.96 (each 3H, s, Me-24, -25, -26, and -27), 1.68 (3H, s, Me-30), 1.88 (1H, m, H-18), 2.01 (3H, s, 3-OAc), 2.06 (3H, s, 23-OAc), 2.22 (1H, m, H-19), 2.98 (1H, m, H-3), 3.69 (1H, d, J = 11.6 Hz, H-23α), 3.83 (1H, d, J = 11.6 Hz, H-23β), 4.28 (2H, m, H-4′), 4.60 (1H, s, H-29α), 4.73 (1H, s, H-29β), 4.77 (2H, m, H-1′), 5.63 (1H, m, H-2′), 5.88 (1H, m, H-3′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 12.8, 14.5, 15.9, 16.5, 19.3, 20.8, 21.1, 23.0, 29.5, 30.5, 33.9, 36.9, 38.1, 40.5, 40.7, 42.3, 46.8, 48.0, 49.4, 50.5, 56.5, 58.4, 59.3, 65.4, 74.4, 109.6, 125.8, 133.3, 150.3, 170.5, 170.9, 176.1.

*4.1.13. 3,23-O-Diacetyl-17-isopropyl betulinic ester (**13**)*

White foam, yield: 77.4%. MS (ESI): *m/z* 599.9 $[M + H]^+$, 621.9 $[M + Na]^+$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.81, 0.88, 0.93, 0.97 (each 3H, s, Me-24, -25, -26, and -27), 1.23 (3H, d, J = 6.2 Hz, Me-2′), 1.24 (3H, d, J = 6.2 Hz, Me-3′), 1.68 (3H, s, Me-30), 1.81 (1H, m, H-18), 2.02 (3H, s, 3-OAc), 2.07 (3H, s, 23-OAc), 2.22 (1H, m, H-19), 3.03 (1H, m, H-3), 3.70 (1H, d, J = 11.6 Hz, H-23α), 3.83 (1H, d, J = 11.6 Hz, H-23β), 4.60 (1H, s, H-29α), 4.75 (1H, s, H-29β), 5.01 (1H, m, H-1′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 12.7, 14.4, 15.8, 16.4, 17.7, 19.2, 20.7, 21.0, 21.6, 23.0, 30.5, 31.9, 36.8, 38.1, 40.4, 40.6, 42.2, 46.9, 47.9, 49.2, 50.4, 56.1, 65.3, 66.6, 74.4, 109.3, 150.5, 170.5, 170.9, 175.4.

*4.1.14. 3,23-O-Diacetyl-28-tert-butyl betulinic ester (**14**)*

White foam, yield: 92.7%. MS (ESI): *m/z* 613.9 $[M + H]^+$, 635.9 $[M + Na]^+$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.80, 0.87, 0.94, 0.95 (each 3H, s, Me-24, -25, -26, and -27), 1.45 (9H, s, Me-2′, -3′, -4′), 1.68 (3H, s, Me-30), 1.81 (1H, m, H-18), 2.01 (3H, s, 3-OAc), 2.06 (3H, s, 23-OAc), 2.22 (1H, m, H-19), 3.03 (1H, m, H-3), 3.68 (1H, d, J = 11.6 Hz, H-23α), 3.83 (1H, d, J = 11.6 Hz, H-23β), 4.59 (1H, s, H-29α), 4.78 (1H, s, H-29β); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 12.8, 14.5, 15.9, 16.5, 19.4, 20.9, 21.1, 23.1, 28.0, 30.7, 32.3, 36.9, 38.0, 38.2, 40.5, 40.7, 42.4, 47.0, 48.0, 49.2, 50.6, 56.8, 65.4, 74.5, 109.3, 150.8, 170.6, 170.9, 175.4.

*4.1.15. 3,23-O-Diacetyl-17-(tetrahydrofufuran-2-yl)methyl betulinic ester (**15**)*

White foam, yield: 80.6%. MS (ESI): *m/z* 641.9 $[M + H]^+$; 663.9 $[M + Na]^+$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.80, 0.87, 0.92, 0.96 (each 3H, s, Me-24, -25, –26, and -27), 1.68 (3H, s, Me-30), 1.81 (1H, m, H-18), 1.90 (4H, m, H-3′, 4′), 2.01 (3H, s, 3-OAc), 2.06 (3H, s, 23-OAc), 2.22 (1H, m, H-19), 3.03 (1H, m, H-3), 3.68 (1H, d, J = 11.6 Hz, H-23α), 3.88 (2H, m, H-5′), 4.04 (1H, m, H-2′), 4.12 (1H, d, J = 11.6 Hz, H-23β), 4.16 (2H, m, H-1′), 4.60 (1H, s, H-29α), 4.73 (1H, s, H-29β); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 12.8, 14.5, 15.9, 16.5, 17.9, 19.3, 20.9, 20.9, 21.1,

23.1, 25.5, 25.6, 28.1, 30.6, 36.9, 38.0, 40.6, 40.7, 42.3, 48.0, 50.6, 56.5, 65.4, 65.7, 68.3, 74.5, 109.5, 150.5, 170.6, 170.9, 175.9.

*4.1.16. 3,23-O-Diacetyl-17-isopentyl betulinic ester (**16**)*

White foam, yield: 63.6%. MS (ESI): *m/z* 543.8 $[M + H]^+$, 565.8 $[M + Na]^+$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.80, 0.87, 0.91, 0.92, 0.94, 0.96 (each 3H, s, Me-24, -25, -26, -27, -4′, -5′), 1.68 (2H, m, H-2′), 1.70 (3H, s, Me-30), 1.86 (1H, m, H-18), 2.01 (3H, s, 3-OAc), 2.06 (3H, s, 23-OAc), 2.24 (1H, m, H-19), 3.03 (1H, m, H-3), 3.68 (1H, d, J = 11.6 Hz, H-23α), 3.83 (1H, d, J = 11.6 Hz, H-23β), 4.07 (2H, m, H-1′), 4.60 (1H, s, H-29α), 4.75 (1H, s, H-29β); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 12.8, 14.5, 15.9, 16.5, 19.3, 20.9, 21.2, 22.3, 22.4, 23.0, 25.1, 36.9, 37.4, 38.2, 40.5, 40.6, 42.3, 46.9, 47.9, 49.3, 50.5, 56.4, 62.4, 65.3, 74.4, 109.5, 150.5, 170.6, 171.0, 176.1.

*4.1.17. N-(3,23-O-Diacetyl-17-betulinic acyl)-L-proline methyl ester (**17**)*

To a solution of compound **3** (0.30 g, 0.52 mmol) in dry dichloromethane (20 mL) was added L-proline methyl ester hydrochloride (0.26 g, 1.56 mmol), DMAP (0.14 g, 1.0 mmol) and dry triethylamine (0.1 mL). The solution was stirred and refluxed for 24 h. At which time the reaction was completed, the solution was concentrated, the residue was dissolved in ethyl acetate (30 mL) and washed by 10% HCl (20 mL) and brine (30 mL), dried with anhydrous Na_2SO_4. Filtered, the filtrate was concentrated, the residue was dissolved in dichloromethane and purified on a silica gel column (200 g, petroleum ether–ethyl acetate 10:1) to give **17** (0.26 g, 74.3.0%) as white foam. MS (ESI): *m/z* 690.6 $[M + Na]^+$; HR-ESI-MS: *m/z* 690.4336 $[M + Na]^+$ (calcd: 690.4340); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.78, 0.84, 0.90, 0.94 (each 3H, s, Me-24, -25, -26, and -27), 1.44 (4H, m, H-3′, 4′), 1.66 (3H, s, Me-30), 1.91 (1H, m, H-18), 1.99 (3H, s, 3-OAc), 2.04 (3H, s, 23-OAc), 2.31 (1H, m, H-19), 3.00 (1H, m, H-3), 3.53 (1H, m, H-2′α), 3.65 (1H, d, J = 11.6 Hz, H-23α), 3.68 (3H, s, OMe-6′), 3.75 (1H, m, H-2′β), 3.82 (1H, d, J = 11.6 Hz, H-23β), 4.44 (1H, t, H-5′), 4.55 (1H, s, H-29α), 4.69 (1H, s, H-29β); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 14.9, 16.6, 18.2, 18.6, 20.0, 21.8, 22.9, 23.0, 23.2, 25.1, 27.7, 36.1, 39.0, 40.0, 42.6, 42.7, 44.1, 47.9, 50.1, 52.8, 54.0, 57.3, 62.2, 67.5, 76.6, 110.9, 153.5, 172.6, 173.0, 175.5, 176.1.

Compounds **18** and **19** were obtained by using the similar synthetic procedure of compound **17**.

*4.1.18. N-(3,23-O-Diacetyl-17-betulinic acyl)-L-glycine methyl ester (**18**)*

White foam, yield: 91.0%. MS (ESI): *m/z* 626.4 $[M - H]^-$, 650.5 $[M + Na]^+$; HR-ESI-MS: *m/z* 626.4040 $[M - H]^-$ (calcd: 626.4062); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.79, 0.86, 0.92, 0.95 (each 3H, s, Me-24, -25, -26, and -27), 1.67 (3H, s, Me-30), 1.96 (1H, m, H-18), 1.99 (3H, s, 3-OAc), 2.04 (3H, s, 23-OAc), 2.41 (1H, m, H-19), 3.07 (1H, m, H-3), 3.66 (1H, d, J = 11.6 Hz, H-23α), 3.73 (3H, s, H-3′–OCH_3), 3.84 (1H, d, J = 11.6 Hz, H-23β), 3.99 (2H, s, H-2′), 4.58 (1H, s, H-29α), 4.71 (1H, s, H-29β), 6.07 (1H, t, H-1′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 12.8, 14.5, 16.0, 16.5, 17.9, 19.4, 20.8, 21.1, 29.3, 36.9, 37.6, 38.0, 40.5, 40.7, 41.0, 42.4, 46.6, 48.0, 50.0, 50.6, 52.1, 55.7, 65.4, 74.5, 109.3, 150.7, 170.6, 170.8, 170.9, 176.5.

*4.1.19. N-(3,23-O-Diacetyl-17-betulinic acyl)-L-methionine methyl ester (**19**)*

White foam, yield: 56.0%. MS (ESI): *m/z* 724.6 $[M + Na]^+$; HR-ESI-MS: *m/z* 700.4249 $[M - H]^-$ (calcd: 700.4252); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.70, 0.83, 0.90, 1.15 (each 3H, s, Me-24, -25, -26, and -27), 1.70 (3H, s, Me-30), 1.93 (2H, m, H-1″), 1.94 (1H, m, H-18), 2.02 (3H, s, Me-4″), 2.06 (3H, s, 3-OAc), 2.09 (3H, s, 23-OAc), 2.11 (2H, m, H-2″), 2.56 (1H, m, H-19), 3.09 (1H, m, H-3), 3.69 (1H, d, J = 11.6 Hz, H-23α), 3.74 (3H, s, OMe-3′), 3.87 (1H, d, J = 11.6 Hz, H-23β), 4.68 (1H, s, H-29α), 4.77 (1H, s, H-29β), 6.61 (1H, d, J = 6.2 Hz, H-1′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 12.6, 15.1, 15.3, 16.1, 20.4, 20.4, 20.7, 23.1, 25.1, 29.5, 30.2, 32.5, 36.2, 38.9, 40.0, 41.4, 41.5, 46.0, 47.2, 51.3, 51.9, 55.3, 64.9, 74.0, 122.7, 143.2, 170.1, 170.4, 172.0, 177.3.

*4.1.20. 3,23-Dihydroxy-17-1,4′-bipiperidinyl betulinic amide (**20**)*

To a solution of compound **4** (0.08 g, 0.11 mmol) in methanol:THF (2:5 mL) was added a NaOH solution (4 mol/L, 2 mL). The solution was stirred and refluxed for 4 h. At which time the reaction was completed, the pH of the solution was adjusted to 4–5 by 10% HCl, filtered, the filter cake was collected and purified on a silica gel column (200 g, 1:1 chloroform-methanol) to give **20** (0.058 g, 82.0%) as white foam. MS (ESI): *m/z* 624.4 $[M + H]^+$; HR-ESI-MS: *m/z* 623.5160 $[M + H]^+$ (calcd: 623.5146); ^{1}H NMR (CD_3OD, 400 MHz) δ: 0.66, 0.88, 0.94, 1.00 (each 3H, s, Me-24, -25, -26, and -27), 1.68 (3H, s, Me-30), 2.02 (1H, m, H-18), 2.15 (1H, m, H-19), 2.18 (4H, m, H-2″, 6″), 2.83 (4H, m, H-2′, 6′), 2.99 (1H, m, H-3), 3.26(1H, d, J = 10.9 Hz, H-23α), 3.50 (1H, d, J = 10.9 Hz, H-23β), 4.57 (1H, s, H-29α), 4.64 (1H, s, H-29β); ^{13}C NMR (CD_3OD, 100 MHz) δ: 13.7, 16.3, 17.9, 18.3, 25.6, 39.3, 43.1, 44.3, 44.6, 49.5, 49.8, 50.0, 50.4, 50.5, 50.6, 50.7, 50.8, 52.5, 53.4, 57.3, 66.1, 68.7, 75.1, 111.1, 153.6, 177.0.

Compounds **21**–**28** were obtained by using the similar synthetic procedure of compound **20**.

*4.1.21. 3,23-Dihydroxy-17-[4-(5-(pyridin-2-yl)-2H-tetrazol-2-yl)-n-butyl]betulinic amide (**21**)*

White foam, yield: 80.0%. MS (ESI): *m/z* 673.6 $[M + H]^+$; HR-ESI-MS: 643.7814 $[M + H]^+$ (calcd: 643.7800); ^{1}H NMR (CD_3OD, 400 MHz) δ: 0.63, 0.78, 0.79, 1.09 (each 3H, s, Me-24, -25, -26, and -27), 2.00 (3H, s, Me-30), 2.11 (1H, m, H-18), 2.17 (1H, m, H-19), 3.21 (1H, m, H-3), 3.49 (1H, d, J = 10.8 Hz, H-23α), 3.58 (1H, m, H-2′α), 3.64 (1H, m, H-2′β), 3.82 (1H, m, H-5′α), 4.81 (1H, m, H-5′β), 4.92 (1H, d, J = 10.8 Hz, H-23β), 4.83 (1H, s, H-29α), 5.47 (1H, s, H-29β), 8.18 (1H, m, H-14′), 8.96 (1H, m, H-15′), 9.12 (1H, d, J = 8.1 Hz, H-16′), 9.51 (1H, s, H-13′); ^{13}C NMR (CD_3OD, 100 MHz) δ: 5.0, 6.6, 8.5, 9.9, 16.4, 20.0, 26.7, 30.5, 33.6, 34.1, 35.8, 40.8, 41.0, 41.1, 41.2, 41.4, 41.7, 41.9, 42.1, 45.0, 59.6, 66.0, 92.5, 121.2, 121.5, 134.2, 134.5, 136.0, 136.3, 137.7, 154.0, 177.0.

*4.1.22. 3,23-Dihydroxy-17-diallyl betulinic amide (**22**)*

White foam, yield: 85.7%. MS (ESI): *m/z* 552.4 $[M + H]^+$; HR-ESI-MS: *m/z* 574.4219 $[M + Na]^+$ (cacld: 574.4231); ^{1}H NMR (pyridine-d_5, 400 MHz) δ: 0.91, 0.98, 1.04, 1.10 (each 3H, s, Me-24, -25, -26, and -27), 1.74 (3H, s, Me-30), 2.05 (1H, m, H-18), 2.16 (1H, m, H-19), 3.24 (1H, m, H-3), 3.70 (1H, d, J = 11.1 Hz, H-23α), 3.95 (2H, m, H-2′), 4.15 (2H, m, H-5′), 4.19 (1H, d, J = 11.1 Hz, H-23β), 4.72 (1H, s, H-29α), 4.87 (1H, s, H-29β), 5.16 (2H, d, J = 8.5 Hz, H-4′), 5.21 (2H, d, J = 12.2 Hz, H-7′), 5.83 (2H, br s, H-3′, 6′); ^{13}C NMR (pyridine-d_5, 100 MHz) δ: 14.3, 16.4, 17.9, 18.3, 20.0, 21.2, 31.7, 33.3, 33.8, 36.0, 37.8, 38.7, 38.8, 40.6, 42.6, 43.7, 44.4, 47.9, 50.4, 52.7, 54.7, 56.5, 69.4, 74.9, 111.0, 118.2, 124.4, 125.4, 136.0, 153.3, 175.9.

*4.1.23. 3,23-Dihydroxy-17-(2-aminoethyl)betulinic amide (**23**)*

White foam, yield: 84.6%. MS (ESI): *m/z* 515.9 $[M + H]^+$; HR-ESI-MS: *m/z* 515.4211 $[M + H]^+$ (cacld: 515.4207); ^{1}H NMR (pyridine-d_5, 400 MHz) δ: 0.94, 1.01, 1.06, 1.15 (each 3H, s, Me-24, -25, -26, and -27), 1.78 (3H, s, Me-30), 2.10 (1H, m, H-18), 2.18 (1H, m, H-19), 3.04 (1H, m, H-3), 3.62 (2H, t, H-2′), 3.72 (1H, d, J = 10.2 Hz, H-23α), 4.18 (1H, d, J = 10.2 Hz, H-23β), 4.76 (1H, s, H-29α), 4.94 (1H, s, H-29β), 5.21 (1H, s, H-1′); ^{13}C NMR (pyridine-d_5, 100 MHz) δ: 13.7, 15.6, 17.4, 17.6, 19.4, 20.4, 27.0, 28.7, 32.2, 35.4, 38.2, 38.6, 39.9, 41.3, 42.0, 43.6, 43.8, 48.1, 49.7, 51.5, 51.9, 56.7, 68.7, 74.3, 110.5, 152.4, 178.3.

*4.1.24. 3,23-Dihydroxy-17-morpholinyl betulinic amide (**24**)*

White foam, yield: 93.8%. MS (ESI): *m/z* 564.7 $[M + Na]^+$; HR-ESI-MS: *m/z* 542.4207 $[M + H]^+$ (cacld: 542.4204); ^{1}H NMR

(pyridine-d_5, 400 MHz) δ: 0.92, 0.99, 1.04, 1.11 (each 3H, s, Me-24, -25, -26, and -27), 1.75 (3H, s, Me-30), 1.92 (1H, m, H-18), 1.99 (1H, m, H-19), 3.35 (1H, m, H-3), 3.62 (8H, m, H-2′, 3′, 5′, 6′), 3.70 (1H, d, J = 10.5 Hz, H-23α), 4.17 (1H, d, J = 10.5 Hz, H-23β), 4.73 (1H, s, H-29α), 4.89 (1H, s, H-29β); ^{13}C NMR (pyridine-d_5, 100 MHz) δ: 12.2, 14.2, 15.8, 16.1, 17.9, 19.0, 20.7, 27.2, 29.5, 31.8, 35.3, 36.5, 36.7, 38.4, 40.4, 41.5, 42.3, 45.7, 48.3, 50.6, 52.3, 54.0, 66.4, 67.3, 72.8, 108.9, 151.0, 172.8.

4.1.25. 3,23-Dihydroxy-17-piperidinyl betulinic amide (**25**)

White foam, yield: 83.9%. MS (ESI): m/z 562.16 $[M + Na]^+$; HR-ESI-MS: m/z 538.4266 $[M - H]^-$ (calcd: 538.4266); ^{1}H NMR (pyridine-d_5, 400 MHz) δ: 0.92, 1.00, 1.02, 1.10 (each 3H, s, Me-24, -25, -26, and -27), 1.75 (3H, s, Me-30), 1.93 (1H, m, H-18), 2.15 (1H, m, H-19), 3.28 (1H, m, H-3), 3.50 (2H, t, H-2′), 3.56 (2H, t, H-6′), 3.68 (1H, d, J = 10.2 Hz, H-23α), 4.13 (1H, d, J = 10.2 Hz, H-23β), 4.87 (1H, s, H-29α), 5.06 (1H, s, H-29β); ^{13}C NMR (pyridine-d_5, 100 MHz) δ: 13.3, 15.3, 16.9, 17.3, 19.0, 20.2, 21.9, 26.9, 28.2, 30.7, 32.2, 35.0, 37.6, 37.8, 39.6, 41.5, 42.7, 43.3, 46.9, 49.4, 51.7, 53.6, 55.3, 68.5, 74.0, 109.9, 152.3, 173.5.

4.1.26. 3,23-Dihydroxy-17-piperizinyl betulinic amide (**26**)

White foam, yield: 76.9%. MS (ESI): m/z 541.5 $[M + H]^+$; HR-ESI-MS: m/z 541.4372 $[M + H]^+$ (calcd: 541.4364); ^{1}H NMR (pyridine-d_5, 400 MHz) δ: 0.90, 0.98, 1.02, 1.10 (each 3H, s, Me-24, -25, -26, and -27), 1.74 (3H, s, Me-30), 2.02 (1H, m, H-18), 2.12 (1H, m, H-19), 2.84 (4H, m, H-3′, 5′), 3.28 (1H, m, H-3), 3.68 (4H, m, H-2′, 6′), 3.73 (1H, d, J = 10.3 Hz, H-23α), 4.16 (1H, d, J = 10.3 Hz, H-23β), 4.72 (1H, s, H-29α), 4.88 (1H, s, H-29β); ^{13}C NMR (pyridine-d_5, 100 MHz) δ: 12.3, 14.2, 15.8, 16.2, 17.9, 19.0, 25.6, 27.2, 29.5, 31.9, 33.9, 36.5, 36.7, 38.5, 40.5, 41.6, 42.3, 45.8, 45.9, 48.2, 50.6, 52.4, 54.1, 67.1, 72.8, 108.9, 151.2, 172.7.

4.1.27. 3,23-Dihydroxy-17-(2-morpholinylethyl)betulinic ester (**27**)

White foam, yield: 85.7%. MS (ESI): m/z 586.6 $[M + H]^+$; HR-ESI-MS: m/z 586.4467 $[M + H]^+$ (cacld: 586.4466); ^{1}H NMR (pyridine-d_5, 400 MHz) δ: 0.90, 0.95, 1.03, 1.05 (each 3H, s, Me-24, -25, -26, and -27), 1.74 (3H, s, Me-30), 2.04 (1H, m, H-18), 2.09 (1H, m, H-19), 2.46 (4H, m, H-4′, 8′), 2.60 (2H, t, H-2′), 3.35 (1H, m, H-3), 3.73 (4H, m, H-5′, 7′), 3.70 (1H, d, J = 11.5 Hz, H-23α), 4.18 (1H, d, J = 11.5 Hz, H-23β), 4.39 (2H, t, H-1′), 4.74 (1H, s, H-29α), 4.89 (1H, s, H-29β); ^{13}C NMR (pyridine-d_5, 100 MHz) δ: 12.4, 14.3, 15.8, 16.2, 18.8, 25.4, 27.3, 29.5, 31.8, 36.7, 37.9, 38.5, 40.5, 42.1, 42.4, 47.0, 48.1, 49.1, 50.3, 53.5, 56.2, 57.0, 60.4, 66.6, 72.7, 86.0, 109.5, 150.4, 175.4.

4.1.28. 3,23-Dihydroxy-17-(E-4-hydroxybut-2-enyl)betulinic ester (**28**)

White foam, yield: 77.8%. MS (ESI): m/z 565.6 $[M + Na]^+$; HR-ESI-MS: m/z 565.3865 $[M + Na]^+$ (cacld: 565.3863); ^{1}H NMR (pyridine-d_5, 400 MHz) δ: 0.90, 0.95, 1.03, 1.07 (each 3H, s, Me-24, -25, -26, and -27), 1.77 (3H, s, Me-30), 2.22 (1H, m, H-18), 2.47 (1H, m, H-19), 3.40 (1H, m, H-3), 3.70 (1H, d, J = 10.5 Hz, H-23α), 4.16 (1H, d, J = 10.5 Hz, H-23β), 4.19 (2H, m, H-4′), 4.60 (1H, s, H-29α), 4.73 (1H, s, H-29β), 5.13 (2H, m, H-1′), 5.81 (1H, m, H-2′), 6.17 (1H, m, H-3′); ^{13}C NMR (pyridine-d_5, 100 MHz) δ: 14.1, 16.0, 17.5, 18.0, 19.7, 20.6, 22.3, 27.2, 29.0, 31.2, 35.6, 38.5, 39.7, 40.3, 42.3, 43.9, 44.2, 48.7, 50.0, 51.0, 52.1, 57.9, 59.6, 61.4, 69.2, 74.7, 111.2, 126.0, 137.1, 152.1, 177.0.

4.1.29. N-(3,23-Dihydroxy-17-betulinic acyl)-L-proline acid (**29**)

To a solution of compound **17** (0.34 g, 0.51 mmol) in methanol:THF (4:10 mL) was added a NaOH solution (4 mol/L, 8 mL). The solution was stirred and refluxed for 4 h. At which time the reaction was completed, the pH of the solution was adjusted to 1–2 by 10% HCl, filtered, the filter cake was collected and dissolved in methanol and purified on a silica gel column (200 g, chloroform–methanol 10:1) to give **29** (0.26 g, 89.7%) as white foam. MS (ESI): m/z 568.6 $[M - H]^-$, 592.4 $[M + Na]^+$; HR-ESI-MS: m/z 568.4010 $[M - H]^-$ (calcd: 568.4007); ^{1}H NMR (pyridine-d_5, 400 MHz) δ: 0.99, 1.03, 1.06, 1.09 (each 3H, s, Me-24, -25, -26, and -27), 1.77 (3H, s, Me-30), 2.03 (1H, m, H-18), 2.08 (4H, m, H-3′, 4′), 2.34 (1H, m, H-19), 3.22 (1H, m, H-3), 3.52 (1H, d, J = 10.5 Hz, H-23α), 3.73 (1H, d, J = 10.5 Hz, H-23β), 3.91 (1H, t, H-5′), 4.64 (1H, s, H-29α), 4.70 (1H, s, H-29β); ^{13}C NMR (pyridine-d_5, 100 MHz) δ: 12.8, 13.1, 14.8, 16.5, 16.8, 18.5, 19.7, 26.1, 27.8, 30.5, 37.2, 37.2, 37.3, 41.0, 42.4, 42.9, 42.9, 48.7, 51.1, 55.3, 55.4, 61.1, 61.3, 67.7, 73.3, 109.2, 151.9, 173.9, 175.6.

Compounds **30** and **31** were obtained by using the similar synthetic procedure of compound **29**.

4.1.30. N-(3,23-Dihydroxy-17-acyl betulinic acyl)-L-glycine acid (**30**)

White foam, yield: 75.0%. MS (ESI): m/z 528.7 $[M - H]^-$; HR-ESI-MS: m/z 528.3674 $[M - H]^-$ (calcd: 528.3694); ^{1}H NMR (CD_3OD, 400 MHz) δ: 0.66, 0.86, 0.94, 0.98 (each 3H, s, Me-24, -25, -26, and -27), 1.66 (3H, s, Me-30), 1.97 (1H, m, H-18), 2.10 (1H, m, H-19), 2.59 (1H, m, H-3), 3.49 (1H, d, J = 12.7 Hz, H-23α), 3.03 (1H, s, H-2′α), 3.68 (1H, s, H-2′β), 3.90 (1H, d, J = 12.7 Hz, H-23β), 4.55 (1H, s, H-29α), 4.67 (1H, s, H-29β); ^{13}C NMR (CD_3OD, 100 MHz) δ: 12.4, 15.0, 17.0, 19.1, 19.6, 22.0, 26.9, 38.0, 38.8, 41.9, 43.5, 47.9, 48.3, 49.2, 49.4, 49.6, 51.9, 56.9, 74.0, 109.8, 152.2, 172.0, 179.7.

4.1.31. N-(3,23-Dihydroxy-17-betulinic acyl)-L-methionine acid (**31**)

White foam, yield: 82.3%. MS (ESI): m/z 602.8 $[M - H]^-$; HR-ESI-MS: m/z 602.3882 $[M - H]^-$ (calcd: 602.3885); ^{1}H NMR (pyridine-d_5, 400 MHz) δ: 0.88, 0.98, 1.03, 1.15 (each 3H, s, Me-24, -25, -26, and -27), 1.73 (3H, s, Me-30), 1.90 (2H, m, H-1″), 2.02 (1H, m, H-18), 2.08 (3H, s, Me-4″), 2.10 (2H, m, H-2″), 2.39 (1H, m, H-19), 2.91 (1H, m, H-3), 3.57 (1H, t, H-2′), 3.70 (1H, d, J = 10.2 Hz, H-23α), 4.17 (1H, d, J = 10.2 Hz, H-23β), 4.72 (1H, s, H-29α), 4.89 (1H, s, H-29β); ^{13}C NMR (pyridine-d_5, 100 MHz) δ: 12.8, 14.8, 15.2, 16.7, 16.8, 19.5, 31.1, 31.3, 34.6, 37.3, 37.7, 39.0, 41.1, 42.7, 42.8, 42.9, 47.1, 48.7, 50.6, 51.0, 55.9, 67.7, 73.3, 110.5, 151.7, 177.6.

4.1.32. 3,23-O-Diacetyl-30-hydroxy-17-isopropyl betulinic ester (**32**)

To a solution of compound **13** (0.34 g, 0.57 mmol) in chloroform (30 mL) was added *m*-CPBA (0.088 g, 0.51 mmol). The solution was stirred and refluxed for 8 h. At which time the reaction was completed, the solution was washed by saturated $NaHCO_3$ solution (40 mL), water (40 mL) and brine (40 mL), dried with anhydrous Na_2SO_4. Filtered, the filtrate was concentrated, the residue was dissolved in dichloromethane and purified on a silica gel column (200 g, petroleum ether–ethyl acetate 5:1) to give **32** (0.16 g, 45.7%) as white foam. MS (ESI): m/z 615.9 $[M + H]^+$, 637.9 $[M + Na]^+$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.79, 0.85, 0.90, 0.95 (each 3H, s, Me-24, -25, -26, and -27), 1.20 (3H, d, J = 6.2 Hz, Me-2′), 1.22 (3H, d, J = 6.2 Hz, Me-3′), 1.81 (1H, m, H-18), 2.00 (3H, s, 3-OAc), 2.05 (3H, s, 23-OAc), 2.22 (1H, m, H-19), 2.89 (1H, m, H-3), 3.68 (1H, d, J = 11.6 Hz, H-23α), 3.82 (1H, d, J = 11.6 Hz, H-23β), 4.10 (2H, s, H-30), 4.75 (1H, m, H-1′), 4.90 (1H, s, H-29α), 4.97 (1H, s, H-29β); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 13.0, 14.7, 16.0, 16.7, 18.0, 21.1, 21.1, 21.3, 21.8, 21.9, 23.2, 29.6, 32.0, 34.0, 38.1, 38.3, 40.7, 48.1, 50.1, 50.6, 65.2, 65.5, 67.0, 74.6, 76.8, 106.7, 155.0, 170.8, 171.2, 175.6.

Compounds **33**–**38** were obtained by using the similar synthetic procedure of compound **32**.

4.1.33. 3,23-O-Diacetyl-30-hydroxy-17-tert-butyl betulinic ester (**33**)

White foam, yield: 55.0%. MS (ESI): m/z 651.6 $[M + Na]^+$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.79, 0.86, 0.92, 0.95 (each 3H, s, Me-24,

-25, -26, and -27), 1.44 (9H, s, Me-2′, -3′, -4′), 1.85 (1H, m, H-18), 2.00 (3H, s, 3-OAc), 2.05 (3H, s, 23-OAc), 2.20 (1H, m, H-19), 2.88 (1H, m, H-3), 3.67 (1H, d, J = 11.6 Hz, H-23α), 3.82 (1H, d, J = 11.6 Hz, H-23β), 4.10 (2H, m, H-30), 4.90 (1H, s, H-29α), 4.95 (1H, s, H-29β); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 13.9, 15.5, 16.9, 17.5, 18.9, 21.9, 22.0, 22.2, 24.1, 29.0, 30.5, 37.9, 37.9, 39.0, 39.1, 41.6, 41.8, 43.3, 49.0, 50.9, 51.6, 57.7, 66.2, 66.4, 75.4, 80.6, 107.5, 156.0, 171.6, 172.0, 176.3.

4.1.34. 3,23-O-Diacetyl-30-hydroxy-17-(tetrahydrofufuran-2-yl) methyl betulinic ester (**34**)

White foam, yield: 59.7%. MS (ESI): m/z 679.8 $[M + Na]^+$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.78, 0.85, 0.89, 0.94 (each 3H, s, Me-24, -25, -26, and -27), 1.66 (4H, m, H-3′, 4′), 1.86 (1H, m, H-18), 2.00 (3H, s, 3-OAc), 2.06 (3H, s, 23-OAc), 2.16 (1H, m, H-19), 3.03 (1H, m, H-3), 3.66 (1H, d, J = 11.6 Hz, H-23α), 3.81 (1H, d, J = 11.6 Hz, H-23β), 4.12 (6H, m, H-1′, 5′, 30), 4.75 (1H, m, H-2′), 4.89 (1H, s, H-29α), 4.95 (1H, s, H-29β); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 13.0, 14.7, 16.0, 16.7, 18.0, 21.0, 21.3, 23.2, 25.8, 32.0, 32.4, 36.8, 37.0, 38.1, 40.6, 40.8, 48.1, 50.6, 65.2, 65.5, 68.4, 74.6, 77.4, 106.7, 154.8, 170.9, 171.2, 175.9.

4.1.35. 3,23-O-Diacetyl-30-hydroxy-17-isopentyl betulinic ester (**35**)

White foam, yield: 31.9%. MS (ESI): m/z 665.9 $[M + Na]^+$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.80, 0.87, 0.91, 0.94, 0.95, 0.96 (each 3H, s, Me-24, -25, -26, -27, -4′, -5′), 1.70 (2H, m, H-2′), 1.85 (1H, m, H-18), 1.87 (1H, m, H-3′), 2.01 (3H, s, 3-OAc), 2.06 (3H, s, 23-OAc), 2.65 (1H, m, H-19), 2.89 (1H, m, H-3), 3.68 (1H, d, J = 11.6 Hz, H-23α), 3.83 (1H, d, J = 11.6 Hz, H-23β), 4.14 (2H, m, H-1′), 4.75 (2H, m, H-30), 4.91 (1H, s, H-29α), 4.97 (1H, s, H-29β); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 12.8, 14.5, 15.9, 16.5, 17.8, 20.9, 21.0, 21.2, 22.3, 22.4, 23.0, 25.1, 36.9, 37.4, 37.9, 38.1, 40.5, 40.6, 42.2, 47.9, 50.0, 50.5, 56.4, 62.4, 65.3, 74.4, 106.5, 154.8, 170.6, 171.0, 176.0.

4.1.36. 3,23-O-Diacetyl-30-hydroxy-17-morpholinyl betulinic amide (**36**)

White foam, yield: 24.5%. MS (ESI): m/z 664.7 $[M + Na]^+$; HR-ESI-MS: m/z 642.4268 $[M + H]^+$ (calcd: 642.4364); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.81, 0.87, 0.92, 0.96 (each 3H, s, Me-24, -25, -26, and -27), 1.95 (1H, m, H-18), 2.02 (3H, s, 3-OAc), 2.07 (3H, s, 23-OAc), 2.20 (1H, m, H-19), 3.56 (1H, m, H-3), 3.70 (4H, m, H-3′, 5′), 3.84 (2H, s, H-30), 4.12 (1H, d, J = 11.6 Hz, H-23α), 4.49 (1H, d, J = 11.6 Hz, H-23β), 4.76 (1H, s, H-29α), 4.95 (1H, s, H-29β); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 12.8, 14.4, 14.6, 16.2, 16.5, 16.6, 17.9, 20.9, 21.2, 23.1, 26.8, 29.7, 36.9, 36.9, 38.0, 40.6, 40.7, 42.0, 48.1, 48.1, 48.9, 50.5, 50.8, 52.3, 60.6, 65.3, 65.4, 74.5, 105.8, 155.8, 170.7, 171.1, 174.1.

4.1.37. 3,23-O-Diacetyl-30-hydroxy-17-piperidinyl betulinic amide (**37**)

White foam, yield: 53.9%. MS (ESI): m/z 640.6 $[M + H]^+$; HR-ESI-MS: m/z 640.4580 $[M + H]^+$ (calcd: 640.4572); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.80, 0.88, 0.94, 0.96 (each 3H, s, Me-24, -25, -26, and -27), 1.95 (1H, m, H-18), 2.01 (3H, s, 3-OAc), 2.06 (3H, s, 23-OAc), 2.10 (1H, m, H-19), 3.03 (1H, m, H-3), 3.49 (4H, m, H-2′, 6′), 3.69 (1H, d, J = 11.6 Hz, H-23α), 3.84 (1H, d, J = 11.6 Hz, H-23β), 4.09 (2H, s, H-30), 4.90 (1H, s, H-29α), 4.94 (1H, s, H-29β); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 12.4, 14.1, 15.6, 16.2, 17.4, 20.5, 20.8, 22.6, 24.3, 25.8, 33.6, 35.3, 36.4, 36.6, 37.6, 40.2, 40.2, 41.4, 47.7, 50.5, 53.2, 54.2, 54.6, 64.8, 65.0, 74.1, 105.3, 155.4, 170.2, 170.6, 172.7.

4.1.38. N-(3,23-O-Diacetyl-30-hydroxy-17-betulinic acyl)-L-proline methyl ester (**38**)

White foam, yield: 69.4%. MS (ESI): m/z 684.7 $[M + H]^+$; HR-ESI-MS: m/z 684.4472 $[M + H]^+$ (calcd: 684.4470); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.81, 0.87, 0.92, 0.97 (each 3H, s, Me-24, -25, -26, and -27), 1.37 (4H, m, H-3′, 4′), 1.91 (1H, m, H-18), 2.01 (3H, s, 3-OAc), 2.07 (3H, s, 23-OAc), 2.74 (1H, m, H-19), 2.92 (1H, m, H-3), 3.56 (2H, m, H-2′), 4.10 (1H, d, J = 11.5 Hz, H-23α), 3.72 (3H, s, H-6′–OCH_3), 3.84 (2H, s, H-30), 4.44 (1H, d, J = 11.5 Hz, H-23β), 4.78 (1H, s, H-29α), 4.92 (1H, s, H-29β); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 12.9, 14.4, 14.6, 16.2, 16.6, 16.6, 17.9, 20.9, 21.2, 23.1, 26.8, 29.7, 36.9, 36.9, 38.0, 40.6, 40.7, 42.0, 48.1, 48.9, 50.5, 50.8, 52.0, 60.1, 65.3, 65.4, 74.5, 105.8, 155.8, 170.7, 171.1, 173.5, 174.1.

4.1.39. 3,23-Dihydroxy-30-hydroxy-17-isopropyl betulinic ester (**39**)

To a solution of compound **32** (0.24 g, 0.39 mmol) in THF (20 mL) were added methanol (8 mL) and NaOH solution (4 mol/L, 8 mL). The solution was stirred and refluxed for 4 h. At which time the reaction was completed, the pH of the solution was adjusted to 5–6 by 10% HCl, filtered, the filter cake was collected and purified on a silica gel column (200 g, 1:1 petroleum ether–acetone) to give **39** (0.18 g, 85.7%) as white foam. MS (ESI): m/z 531.9 $[M + H]^+$, 553.8 $[M + Na]^+$; HR-ESI-MS: m/z 553.3865 $[M + Na]^+$ (calcd: 553.3863); ^{1}H NMR (pyridine-d_5, 400 MHz) δ: 0.90, 0.95, 1.02, 1.04 (each 3H, s, Me-24, -25, -26, and -27), 1.20 (3H, d, J = 6.2 Hz, Me-2′), 1.24 (3H, d, J = 6.2 Hz, H-3′-Me), 1.91 (1H, m, H-18), 2.19 (1H, m, H-19), 3.32 (1H, m, H-3), 3.70 (1H, d, J = 10.6 Hz, H-23α), 4.17 (1H, d, J = 10.6 Hz, H-23β), 4.52 (2H, s, H-30), 5.12 (1H, s, H-29α), 5.16 (1H, m, H-1′), 5.49 (1H, s, H-29β); ^{13}C NMR (pyridine-d_5, 100 MHz) δ: 13.0, 14.9, 16.4, 16.9, 18.5, 21.3, 21.8, 21.8, 27.9, 30.1, 34.5, 37.1, 37.3, 38.6, 39.2, 41.1, 42.7, 43.0, 48.6, 50.2, 50.9, 56.2, 56.6, 64.5, 67.0, 67.6, 73.2, 106.2, 156.8, 175.5.

Compounds **40**–**42** were obtained by using the similar synthetic procedure of compound **39**.

4.1.40. 3,23-Dihydroxy-30-hydroxy-17-tert-butyl betulinic ester (**40**)

White foam, yield: 82.4%. MS (ESI): m/z 567.4 $[M + Na]^+$; HR-ESI-MS: m/z 567.4023 $[M + Na]^+$ (cacld: 567.4020); ^{1}H NMR (pyridine-d_5, 400 MHz) δ: 0.94, 0.98, 1.06, 1.06 (each 3H, s, Me-24, -25, -26, and -27), 1.53 (9H, s, Me-2′, 3′, 4′), 2.11 (1H, m, H-18), 2.48 (1H, m, H-19), 3.30 (1H, m, H-3), 3.72 (1H, d, J = 10.3 Hz, H-23α), 4.18 (1H, d, J = 10.3 Hz, H-23β), 4.49 (2H, m, H-30), 5.13 (1H, s, H-29α), 5.50 (1H, s, H-29β); ^{13}C NMR (pyridine-d_5, 100 MHz) δ: 13.1, 15.1, 16.6, 17.1, 21.5, 27.4, 28.1, 28.3, 30.2, 30.3, 32.8, 34.7, 37.6, 38.8, 39.4, 41.4, 42.9, 43.2, 43.6, 49.0, 50.4, 51.2, 57.3, 64.8, 68.1, 73.7, 79.6, 106.3, 157.2, 175.6.

4.1.41. 3,23-Dihydroxy-30-hydroxy-17-(tetrahydrofufuran-2-yl) methyl betulinic ester (**41**)

White foam, yield: 78.3%. MS (ESI): m/z 573.9 $[M + H]^+$, 595.8 $[M + Na]^+$; HR-ESI-MS: m/z 595.3968 $[M + Na]^+$ (cacld: 595.3969); ^{1}H NMR (pyridine-d_5, 400 MHz) δ: 0.61, 0.67, 0.75, 0.77 (each 3H, s, Me-24, -25, -26, and -27), 1.67 (4H, m, H-3′, 4′), 1.86 (1H, m, H-18), 2.16 (1H, m, H-19), 3.06 (1H, m, H-3), 3.43 (2H, m, H-5′), 3.56 (1H, d, J = 14.4 Hz, H-23α), 3.92 (4H, m, H-1′, 30), 4.07 (1H, m, H-2′), 4.25 (1H, d, J = 14.4 Hz, H-23β), 4.83 (1H, s, H-29α), 5.21 (1H, s, H-29β); ^{13}C NMR (pyridine-d_5, 100 MHz) δ: 13.0, 14.9, 16.3, 16.8, 18.5, 21.2, 26.0, 27.8, 28.3, 32.3, 32.8, 34.5, 37.1, 37.3, 39.1, 41.1, 42.7, 43.0, 48.7, 50.2, 50.9, 56.9, 64.5, 67.7, 68.3, 73.3, 76.9, 77.7, 99.0, 106.2, 156.7, 176.0.

4.1.42. 3,23-Dihydroxy-30-hydroxy-17-isopentyl betulinic ester (**42**)

White foam, yield: 81.2%. MS (ESI): m/z 581.6 $[M + Na]^+$; HR-ESI-MS: m/z 581.4168 $[M + Na]^+$ (cacld: 581.4176); ^{1}H NMR (pyridine-d_5, 400 MHz) δ: 0.88, 0.90, 0.96, 0.99, 1.06, 1.07 (each 3H, s, Me-24, -25, -26, -27, -4′, -5′), 1.74 (2H, m, H-2′), 1.94 (1H, m, H-18), 1.95 (1H, m, H-19), 2.01 (1H, m, H-3′), 3.31 (1H, m, H-3), 3.71 (1H, d, J = 10.3 Hz, H-23α), 4.17 (1H, d, J = 10.3 Hz, H-23β), 4.31 (2H, m, H-1′), 4.53 (2H, m, H-30), 5.14 (1H, s, H-29α), 5.50 (1H, s, H-29β);

^{13}C NMR (pyridine-d_5, 100 MHz) δ: 14.1, 16.1, 17.6, 18.0, 19.8, 22.5, 23.7, 26.6, 29.1, 31.4, 35.8, 38.6, 39.0, 39.9, 40.4, 42.4, 44.0, 44.2, 50.1, 51.5, 52.2, 58.1, 63.8, 65.8, 69.2, 69.8, 74.7, 107.5, 158.0, 177.2.

4.1.43. N-[N-(3,23-Dihydroxy-17-betulinic acyl)-L-glycine acyl]-L-alanine methyl ester (**43**)

To a solution of compound **30** (0.40 g, 0.75 mmol) in dry dichloromethane (50 mL) were added L-alanine methyl ester hydrochloride (0.42 g, 3.0 mmol), EDC·HCl (1.15 g, 6.0 mmol) and HoBt (0.82 g, 6.0 mmol). The solution was stirred and refluxed for 10 h. At which time the reaction was completed, the solution was washed by water (100 mL) and brine (100 mL), dried with anhydrous Na_2SO_4. Filtered, the filtrate was concentrated, the residue was dissolved in dichloromethane and purified on a silica gel column (200 g, petroleum ether–acetone 3:1) to give **43** (0.33 g, 72.0%) as white foam. MS (ESI): m/z 637.6 $[M + Na]^+$; HR-ESI-MS: m/z 613.4214 $[M - H]^-$ (calcd: 613.4222); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.84, 0.84, 0.88, 0.94 (each 3H, s, Me-24, -25, -26, and -27), 1.40 (3H, s, Me-5′), 1.64 (3H, s, Me-30), 1.81 (1H, m, H-18), 2.43 (1H, m, H-19), 3.05 (1H, m, H-3), 3.38 (1H, d, J = 10.3 Hz, H-23α), 3.69 (1H, d, J = 10.3 Hz, H-23β), 3.72 (3H, s, OMe-6′), 3.90 (2H, d, J = 5.2 Hz, H-2′), 4.52 (1H, m, H-5′), 4.58 (1H, s, H-29α), 4.71 (1H, s, H-29β), 6.51 (1H, t, H-1′), 6.86 (1H, d, J = 7.0 Hz, H-4′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 11.2, 14.6, 16.1, 16.4, 18.1, 18.3, 19.4, 30.7, 37.0, 37.6, 40.7, 41.8, 42.4, 43.1, 46.6, 48.1, 49.9, 50.0, 50.5, 52.4, 55.8, 71.8, 76.6, 109.4, 150.6, 169.1, 173.0, 177.2.

Compounds **44**–**47** were obtained by using the similar synthetic procedure of compound **43**.

4.1.44. N-[N-(3,23-Dihydroxy-17-betulinic acyl)-L-glycine acyl]-L-proline methyl ester (**44**)

White foam, yield: 50.0%. MS (ESI): m/z 639.4 $[M - H]^-$, 663.6 $[M + Na]^+$; HR-ESI-MS: m/z 639.4371 $[M - H]^-$ (calcd: 639.4379); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.79, 0.81, 0.89, 0.94 (each 3H, s, Me-24, -25, -26, and -27), 1.66 (3H, s, Me-30), 1.87 (1H, m, H-18), 2.04 (4H, m, H-6′, 7′), 2.43 (1H, m, H-19), 3.05 (1H, m, H-3), 3.39 (1H, d, J = 11.0 Hz, H-23α), 3.53 (2H, m, H-5′), 3.65 (1H, d, J = 11.0 Hz, H-23β), 3.75 (3H, s, OMe-9′), 3.90 (1H, m, H-2′α), 4.15 (1H, m, H-2′β), 4.52 (1H, m, H-8′), 4.58 (1H, s, H-29α), 4.71 (1H, s, H-29β), 6.60 (1H, t, H-1′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 11.2, 14.5, 16.1, 16.4, 18.3, 19.3, 24.5, 29.0, 37.0, 37.5, 40.6, 41.7, 41.8, 42.4, 45.9, 46.6, 49.9, 50.0, 50.5, 52.4, 55.7, 58.8, 76.6, 109.3, 150.8, 167.5, 172.3, 176.7.

4.1.45. N-[N-(3,23-Dihydroxy-17-betulinic acyl)-L-methionine acyl]-L-methionine methyl ester (**45**)

White foam, yield: 83.0%. MS (ESI): m/z 750.1 $[M + H]^+$; HR-ESI-MS: m/z $[M - H]^-$ 747.4735 (calcd: 747.4446); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.85, 0.86, 0.88, 0.95 (each 3H, s, Me-24, -25, -26, and -27), 1.67 (3H, s, Me-30), 1.98 (1H, m, H-18), 2.09 (3H, s, Me-4″), 2.13 (3H, s, Me-8″), 2.41 (1H, m, H-19), 2.50 (4H, m, H-1″, 5″), 2.64 (4H, m, H-2″, 6″), 3.10 (1H, m, H-3), 3.40 (1H, d, J = 10.3 Hz, H-23α), 3.70 (1H, d, J = 10.3 Hz, H-23β), 3.75 (3H, s, Me-6′), 4.55 (1H, s, H-29α), 4.64 (2H, m, H-2′, 5′), 4.73 (1H, s, H-29β); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 11.2, 14.6, 15.1, 15.4, 16.1, 16.4, 19.4, 29.9, 30.0, 30.2, 31.4, 37.1, 40.7, 41.9, 42.5, 49.9, 50.6, 51.5, 51.6, 52.5, 55.8, 76.5, 76.6, 109.5, 150.6, 171.3, 171.8, 176.5.

4.1.46. N-[N-(3,23-Dihydroxy-17-betulinic acyl)-L-proline acyl]-L-methionine methyl ester (**46**)

White foam, yield: 64.0%. MS (ESI): m/z 716.0 $[M + H]^+$, 737.8 $[M + Na]^+$; HR-ESI-MS: m/z $[M - H]^-$ 713.4563 (calcd: 713.4569); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.84, 0.85, 0.88, 0.94 (each 3H, s, Me-24, -25, -26, and -27), 1.42 (4H, m, H-3′, 4′), 1.67 (3H, s, Me-30), 2.05 (1H, m, H-18), 2.08 (3H, s, Me-4″), 2.19 (2H, m, H-1″), 2.50 (2H, m, H-2″), 2.73 (1H, m, H-19), 2.95 (1H, m, H-3), 3.40 (1H, d, J = 10.2 Hz, H-23α), 3.62 (2H, t, H-2′), 3.70 (1H, d, J = 10.2 Hz, H-23β), 3.74 (3H, s, Me-9′), 4.55 (1H, s, H-29α), 4.61 (1H, m, H-5′), 4.71 (1H, s, H-29β); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 11.2, 14.7, 15.4, 16.1, 16.5, 18.4, 19.6, 21.0, 25.6, 29.9, 36.9, 37.1, 38.4, 40.6, 41.8, 42.1, 46.0, 50.0, 50.8, 51.7, 52.3, 52.4, 55.5, 72.0, 76.6, 76.8, 109.2, 151.2, 172.2, 172.5, 175.1.

4.1.47. N-[N-(3,23-Dihydroxy-17-betulinic acyl)-L-glycine acyl]-L-tryptophane methyl ester (**47**)

White foam, yield: 55.9%. MS (ESI): m/z 730.3 $[M + H]^+$; HR-ESI-MS: m/z 752.4582 $[M + Na]^+$ (calcd: 752.4606); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.80, 0.83, 0.87, 0.91 (each 3H, s, Me-24, -25, -26, and -27), 1.64 (3H, s, Me-30), 1.75 (1H, m, H-18), 2.34 (1H, m, H-19), 3.00 (1H, m, H-3), 3.31 (2H, m, H-1″), 3.38 (1H, d, J = 11.2 Hz, H-23α), 3.70 (3H, s, Me-6′), 3.77 (2H, m, H-2′), 3.95 (1H, d, J = 11.2 Hz, H-23β), 4.57 (1H, s, H-29α), 4.69 (1H, s, H-29β), 4.92 (1H, m, H-5′), 6.97 (1H, d, J = 2.0 Hz, H-4″), 7.09 (1H, t, H-7″), 7.18 (1H, t, H-8″), 7.34 (1H, s, H-3″), 7.42 (1H, d, J = 8.0 Hz, H-6″), 7.52 (1H, d, J = 7.8 Hz, H-9″); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 14.5, 15.9, 16.4, 19.3, 23.1, 27.5, 27.5, 29.6, 30.6, 37.0, 37.6, 38.3, 40.6, 41.8, 42.4, 42.8, 46.6, 49.9, 52.3, 52.4, 53.1, 55.7, 76.6, 76.8, 109.4, 111.2, 116.9, 118.3, 119.6, 122.2, 126.4, 128.4, 136.1, 150.6, 171.8, 172.3, 177.1.

4.1.48. N-[N-(3,23-Dihydroxy-17-betulinic acyl)-L-glycine acyl]-L-alanine acid (**48**)

To a solution of compound **43** (0.29 g, 0.47 mmol) in methanol:THF (20:20 mL) was added a NaOH solution (4 mol/L, 10 mL). The solution was stirred and refluxed for 4 h. At which time the reaction was completed, the pH of the solution was adjusted to 1–2 by 10% HCl, filtered, the filter cake was collected and dissolved in methanol and purified on a silica gel column (200 g, petroleum ether–acetone 1:1) to give **48** (0.25 g, 89.0%) as white foam. MS (ESI): m/z 599.5 $[M - H]^-$; HR-ESI-MS: m/z 599.4059 $[M - H]^-$ (calcd: 599.4066); ^{1}H NMR (pyridine-d_5, 400 MHz) δ: 0.77, 0.89, 0.90, 1.06 (each 3H, s, Me-24, -25, -26, and -27), 1.41 (3H, s, Me-5′), 1.66 (3H, s, Me-30), 2.20 (2H, m, H-18, 19), 2.86 (1H, m, H-3), 3.51 (1H, d, J = 10.1 Hz, H-23α), 3.59 (1H, d, J = 10.1 Hz, H-23β), 4.18 (2H, d, J = 5.2 Hz, H-2′), 4.38 (1H, m, H-5′), 4.65 (1H, s, H-29α), 4.78 (1H, s, H-29β); ^{13}C NMR (pyridine-d_5, 100 MHz) δ: 13.3, 15.2, 16.9, 17.2, 18.9, 19.9, 21.6, 26.6, 30.2, 33.9, 37.6, 39.5, 41.5, 42.0, 43.1, 43.3, 47.6, 48.8, 50.0, 51.4, 52.5, 56.5, 67.5, 73.5, 110.1, 152.1, 172.1, 174.4, 178.6.

Compounds **49**–**52** were obtained by using the similar synthetic procedure of compound **48**.

4.1.49. N-[N-(3,23-Dihydroxy-17-betulinic acyl)-L-glycine acyl]-L-proline acid (**49**)

White foam, yield: 86.2%. MS (ESI): m/z 625.6 $[M - H]^-$; HR-ESI-MS: m/z 625.4220 $[M - H]^-$ (calcd: 625.4222); ^{1}H NMR (pyridine-d_5, 400 MHz) δ: 0.88, 0.99, 1.03, 1.11 (each 3H, s, Me-24, -25, -26, and -27), 1.70 (3H, s, Me-30), 1.77 (1H, m, H-18), 1.86 (4H, m, H-3′, 4′), 2.12 (2H, m, H-6′), 2.28 (1H, m, H-19), 2.41 (2H, m, H-7′), 2.97 (1H, m, H-3), 3.32 (2H, m, H-2′), 3.67 (1H, d, J = 11.3 Hz, H-23α), 4.01 (1H, d, J = 11.3 Hz, H-23β), 4.41 (1H, m, H-8′), 4.67 (1H, s, H-29α), 4.78 (1H, s, H-29β); ^{13}C NMR (pyridine-d_5, 100 MHz) δ: 12.2, 13.5, 14.2, 16.0, 16.2, 18.0, 18.9, 18.9, 25.6, 27.1, 36.7, 37.1, 38.5, 40.6, 42.2, 42.2, 42.3, 46.5, 48.2, 50.5, 55.5, 59.6, 61.4, 67.3, 72.9, 108.9, 151.1, 176.5.

4.1.50. N-[N-(3,23-Dihydroxy-17-betulinic acyl)-L-methionine acyl]-L-methionine acid (**50**)

White foam, yield: 92.0%. MS (ESI): m/z 733.9 $[M - H]^-$, 757.6 $[M + Na]^+$; HR-ESI-MS: m/z $[M - H]^-$ 733.4280 (calcd: 733.4290); ^{1}H NMR (CD_3OD, 400 MHz) δ: 0.87, 0.90, 0.95, 1.00 (each 3H, s, Me-24, -25, -26, and -27), 1.76 (3H, s, Me-30), 1.97 (1H, m, H-18), 2.08 (3H, s, Me-4″), 2.08 (3H, s, Me-8″), 2.19 (1H, m, H-19), 2.54 (8H, m, H-1″, 2″, 5″, 6″), 3.03 (1H, m, H-3), 3.50 (1H, d, J = 11.2 Hz, H-23α),

3.59 (1H, d, J = 11.2 Hz, H-23β), 4.49 (1H, s, H-29α), 4.57 (2H, m, H-2′, 5′), 4.69 (1H, s, H-29β); ^{13}C NMR (CD_3OD, 100 MHz) δ: 12.5, 15.0, 15.2, 15.3, 17.1, 19.1, 38.1, 41.9, 43.3, 43.6, 48.3, 48.5, 48.7, 49.2, 49.4, 49.6, 52.0, 57.1, 67.6, 74.0, 109.9, 152.1, 174.3, 174.6, 179.0.

4.1.51. N-[N-(3,23-Dihydroxy-17-betulinic acyl)-L-proline acyl]-L-methionine acid (**51**)

White foam, yield: 80.0%. MS (ESI): m/z 699.9 $[M - H]^-$, 701.9 $[M + H]^+$; HR-ESI-MS: m/z 723.4383 $[M + Na]^+$(calcd: 727.4377); ^{1}H NMR (pyridine-d_5, 400 MHz) δ: 0.97, 1.02, 1.06, 1.17 (each 3H, s, Me-24, -25, -26, and -27), 1.80 (3H, s, Me-30), 1.97 (4H, m, Me-3′, 4′), 2.05 (1H, m, H-18), 2.11 (3H, s, Me-4″), 2.15 (1H, m, H-19), 2.31 (4H, m, H-1″, 2″), 3.05 (1H, m, H-3), 3.72 (1H, d, J = 11.4 Hz, H-23α), 4.19 (1H, d, J = 11.4 Hz, H-23β), 4.22 (2H, t, H-2′), 4.76 (1H, s, H-29α), 4.95 (1H, m, H-5′), 4.99 (1H, s, H-29β); ^{13}C NMR (pyridine-d_5, 100 MHz) δ: 12.8, 14.8, 15.1, 16.6, 16.8, 18.5, 19.8, 21.4, 26.2, 27.8, 29.8, 30.7, 34.5, 37.2, 37.3, 39.1, 41.1, 42.4, 42.9, 48.8, 51.1, 55.4, 61.7, 67.9, 73.4, 109.4, 152.0, 174.1.

4.1.52. N-[N-(3,23-Dihydroxy-17-betulinic acyl)-L-glycine acyl]-L-tryptophane acid (**52**)

White foam, yield: 58.4%. MS (ESI): m/z 715.0 $[M - H]^-$; HR-ESI-MS: m/z 714.4482 $[M - H]^-$ (calcd: 714.4488); ^{1}H NMR (CD_3OD, 400 MHz) δ: 0.65, 0.81, 0.83, 0.95 (each 3H, s, Me-24, -25, -26, and -27), 1.67 (3H, s, Me-30), 2.09 (1H, m, H-18), 2.47 (1H, m, H-19), 3.19 (1H, m, H-3), 3.27 (1H, d, J = 10.8 Hz, H-23α), 3.49 (1H, d, J = 10.8 Hz, H-23β), 3.55 (2H, m, H-1″), 3.84 (2H, m, H-2′), 4.55 (1H, s, H-29α), 4.57 (1H, m, H-5′), 4.67 (1H, s, H-29β), 6.97 (1H, t, H-7″), 7.04 (1H, t, H-8″), 7.09 (1H, s, H-3″), 7.27 (1H, d, J = 8.0 Hz, H-6″), 7.54 (1H, d, J = 7.8 Hz, H-9″); ^{13}C NMR (CD_3OD, 100 MHz) δ: 10.6, 15.2, 21.5, 36.2, 40.0, 41.4, 46.5, 46.7, 46.9, 47.1, 47.2, 47.2, 47.4, 47.4, 47.5, 47.6, 47.7, 55.2, 65.7, 72.1, 108.1, 109.8, 110.3, 117.6, 117.8, 120.4, 122.4, 127.5, 136.1, 150.4, 169.8, 177.7.

4.1.53. 1,4′-Dipiperidine-1-carbonyl chloride

To a solution of triphosgene (8 g, 26.9 mmol) in dry dichloromethane (40 mL) at −7 °C were added dry triethylamine (1 mL, 7.1 mmol) and dropwise a solution of 1,4′-dipiperidine (5 g, 29.7 mmol) in dry dichloromethane (35 mL) over a period of 50 min. Then the solution was stirred at room temperature for 8 h, at which time the reaction was completed, the solution was filtered, the filtrate was concentrated, the residue was reacted with 23-hydroxybetulinic acid directly without further purification.

4.1.54. 3-Hydroxy-23-O-(1,4′-bipiperidine-1-carbonyl)betulinic acid (**53**)

To a solution of 1,4′-dipiperidine-1-carbonyl chloride mixture obtained from previous reaction in dry pyridine (120 mL) was added 23-hydroxybetulinic acid (1.0 g, 2.1 mmol). The solution was stirred at room temperature for 16 h. At which time the reaction was completed, ethyl acetate (100 mL) was added, the pH of the solution was adjusted to 4–5 by 10% HCl, the organic layer was separated and washed by brine (100 mL), dried with anhydrous Na_2SO_4. Filtered, the filtrate was concentrated, the residue was dissolved in methanol and purified on a silica gel column (200 g, chloroform–methanol 5:1) to give **53** (1.2 g, 85.7%) as white foam. MS (ESI): m/z 667.5 $[M + H]^+$, 665.9 $[M - H]^-$; HR-ESI-MS: m/z 667.5056 $[M + H]^+$ (calcd: 667.5044); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.51, 0.78, 0.86, 0.94 (each 3H, s, Me-24, -25, -26, and -27), 1.36 (6H, H-3″,4″,5″), 1.46 (4H, H-3′, 5′), 1.66 (3H, s, Me-30), 1.92 (1H, m, H-18), 2.12 (1H, m, H-19), 2.42 (2H, t, H-2″), 2.50 (2H, t, H-6″), 2.86 (2H, t, H-2′), 3.02 (2H, t, H-6′), 3.06 (1H, m, H-3), 3.84 (1H, d, J = 11.6 Hz, H-23α), 4.01 (1H, d, J = 11.6 Hz, H-23β), 4.58 (1H, s, H-29α), 4.71 (1H, s, H-29β); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 12.3, 13.4, 14.2, 18.5, 18.8, 24.4, 26.0, 29.9, 36.3, 38.8, 39.2, 39.5, 39.7, 39.9, 40.1, 41.8, 42.0, 46.6, 49.5, 56.4, 60.7, 64.9, 70.2, 109.9, 149.6, 166.8, 171.6.

4.1.55. 3,23-Dioxylcarbonyl-17-1,4′-bipiperidinyl betulinic amide (**54**)

The synthesis of compound **54** has been discussed in Scheme 4 and Fig. 2. MS (ESI): m/z 650.0 $[M + H]^+$; HR-ESI-MS: m/z 649.4940 $[M + H]^+$ (calcd: 649.4949); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.85, 0.85, 0.90, 0.92 (each 3H, s, Me-24, -25, -26, and -27), 1.48 (3H, s, Me-30), 1.86 (8H, H-2′,2″,6′,6″), 2.02 (1H, m, H-18), 1.95 (1H, m, H-19), 2.96 (1H, m, H-3), 3.76 (1H, d, J = 7.2 Hz, H-23α), 3.98 (1H, d, J = 7.2 Hz, H-23β), 4.52 (1H, s, H-29α), 4.72 (1H, s, H-29β); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 11.2, 14.6, 16.1, 16.4, 19.4, 19.6, 21.1, 24.6, 25.6, 26.2, 29.0, 29.9, 31.4, 35.9, 36.8, 37.1, 40.6, 41.8, 41.9, 45.6, 50.4, 52.7, 54.6, 62.7, 70.5, 73.3, 76.7, 109.0, 151.4, 173.2.

4.1.56. 3,23-O-Diacetyl-17-(2-(3-carboxypropanamido) ethylcarbamoyl)betulinic amide (**55**)

To a solution of compound **7** (0.15 g, 0.25 mmol) in dry dichloromethane (40 mL) was added succinic anhydride (0.084 g, 0.84 mmol), DMAP (0.10 g, 0.84 mmol). The solution was stirred and refluxed for 6 h. At which time the reaction was completed, the solution was washed by water (40 mL) and brine (40 mL), dried with anhydrous Na_2SO_4. Filtered, the filtrate was concentrated, the residue was dissolved in dichloromethane and purified on a silica gel column (200 g, 1:1 petroleum ether–acetone) to give **55** (0.13 g, 72.2%) as white foam. MS (ESI): m/z 700.0 $[M + H]^+$, 697.9 $[M - H]^-$, 721.9 $[M + Na]^+$; HR-ESI-MS: m/z 721.4402 $[M + Na]^+$ (calcd: 721.4398); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.82, 0.88, 0.93, 0.97 (each 3H, s, Me-24, -25, -26, and -27), 1.69 (3H, s, Me-30), 1.98 (1H, m, H-18), 2.03 (3H, s, 3-OAc), 2.07 (3H, s, 23-OAc), 2.45 (1H, m, H-19), 2.53 (2H, t, H-2′), 2.69 (2H, t, H-3′), 3.07 (1H, m, H-3), 3.39 (4H, m, H-6′, 7′), 3.69 (1H, d, J = 11.7 Hz, H-23α), 3.85 (1H, d, J = 11.7 Hz, H-23β), 4.61 (1H, s, H-29α), 4.77 (1H, s, H-29β); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 12.5, 14.1, 15.7, 16.2, 17.5, 18.9, 20.5, 20.8, 29.5, 30.4, 36.6, 37.3, 40.2, 40.3, 42.0, 46.3, 47.6, 49.6, 50.2, 55.3, 65.0, 74.1, 109.1, 150.2, 170.2, 170.6, 172.9, 174.8, 177.8.

4.2. Antiproliferative activity

4.2.1. Cell culture

The human breast carcinoma cell line MCF-7 and human hepatocellular carcinoma cell line HepG2 were purchased from American Type Culture Collection; the human cervical carcinoma cell line Hela, human melanoma cell line A375 and mice melanoma cell line B16 were obtained from Chinese Academy of Sciences Committee Type Culture Collection. All cell lines were cultured in RPMI 1640 (Gibco) containing 10% fetal bovine serum (Gibco) and 1% penicillin streptomycin (Gibco) at 37 °C in the presence of 5% CO_2.

4.2.2. Viability assay

MTT assay was used to evaluate the antiproliferative activity of these compounds. Briefly, cells were seeded in 96-well plates overnight. All of the reported 23-hydroxybetulinic acid derivatives were dissolved in DMSO while the positive control doxorubicin was dissolved in PBS. These tested compounds at different concentrations were added into wells and cells were treated at 37 °C for 72 h. Then MTT (5 mg/mL, in PBS) was added into each well and cultured for another 4 h. The optical density was detected in a microplate reader at 570 nm. IC_{50} values were calculated according to the dose-dependent curves.

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Appendix. Supplementary material

Supplementary material associated with this article can be found in online version at doi:10.1016/j.ejmech.2011.03.038.

References

[1] I. Sun, H.K. Wang, Y. Kashiwada, J.K. Shen, L.M. Cosentino, C.H. Chen, L.M. Yang, K.H. Lee, J. Med. Chem. 41 (1998) 4648–4657.
[2] K. Nakagawa-Goto, K. Yamada, M. Taniguchi, H. Tokuda, K.H. Lee, Bioorg. Med. Chem. Lett. 19 (2009) 3378–3381.
[3] K. Qian, D. Yu, C.H. Chen, L. Huang, S.L. Morris-Natschke, T.J. Nitz, K. Salzwedel, M. Reddick, G.P. Allaway, K.H. Lee, J. Med. Chem. 52 (2009) 3248–3258.
[4] Z. Dang, W. Lai, K. Qian, P. Ho, K.H. Lee, C.H. Chen, L. Huang, J. Med. Chem. 52 (2009) 7887–7891.
[5] R. Cusuk, A. Barthel, R. Kluge, D. Strohl, H. Kommera, R. Paschke, Bioorg. Med. Chem. 18 (2010) 1344–1355.
[6] V. Kumar, N. Rani, P. Aggarwal, V.K. Sanna, A.T. Singh, M. Jaggi, N. Joshi, P.K. Sharma, R. Irchhaiya, A.C. Burman, Bioorg. Med. Chem. Lett. 18 (2008) 5058–5062.
[7] H. Kommera, G.N. Kaluderovic, J. Kalbitz, R. Paschke, Invest. New Drugs 29 (2011) 266–272.
[8] H. Kommera, G.N. Kaluderovic, S. Dittrich, J. Kalbitz, B. Dräger, T. Mueller, R. Paschke, Bioorg. Med. Chem. Lett. 20 (2010) 3409–3412.
[9] R.C. Santos, J.A.R. Salvador, S. Marín, M. Cascante, J.N. Moreira, T.C.P. Dinies, Bioorg. Med. Chem. 18 (2010) 4385–4396.
[10] D. Gerrish, I.C. Kim, D.V. Kumar, H. Austin, J.E. Garrus, V. Baichwal, M. Saunders, R.S. McKinnon, M.B. Anderson, R. Carlson, E. Arranz-Plaza, K.M. Yager, Bioorg. Med. Chem. Lett. 18 (2008) 6377–6380.
[11] R.C. Santos, J.A.R. Salvador, S. Marin, M. Cascante, Bioorg. Med. Chem. 17 (2009) 6241–6250.
[12] M. Drag-Zalesinska, J. Kulbacka, J. Saczko, T. Wysocka, M. Zabel, P. Surowiak, M. Drag, Bioorg. Med. Chem. Lett. 19 (2009) 4814–4817.
[13] M. Kvasnica, J. Sarek, E. Klinotova, P. Dzubak, M. Hajduch, Bioorg. Med. Chem. 13 (2005) 3447–3454.
[14] R. Csuk, A. Barthel, S. Schwarz, H. Kommera, R. Paschke, Bioorg. Med. Chem. 18 (2010) 2549–2558.
[15] H. Kommera, G.N. Kaluderouvić, J. Kalbitz, B. Dräger, R. Paschke, Eur. J. Med. Chem. 45 (2010) 3346–3353.
[16] L. Pohjala, S. Alakurtti, T. Ahola, J. Yli-Kauhaluoma, P. Tammela, J. Nat. Prod. 72 (2009) 1917–1926.
[17] M. Urban, J. Sarek, I. Tislerova, P. Dzubak, M. Hajduch, Bioorg. Med. Chem. 13 (2005) 5527–5535.
[18] A. Koohang, N.D. Majewski, E.L. Szotek, A.A. Mar, D.A. ESIznhamer, M.T. Flavin, Z.Q. Xu, Bioorg. Med. Chem. Lett. 19 (2009) 2168–2171.
[19] F. Hashimoto, Y. Kashiwada, L.M. Cosentino, C.-H. Chen, P.E. Garrett, K.-H. Lee, Bioorg. Med. Chem. 12 (1997) 2133–2143.
[20] L. Huang, P. Ho, K.H. Lee, C.H. Chen, Bioorg. Med. Chem. 14 (2006) 2279–2289.
[21] P. Zhu, Y. Bi, J. Xu, Z. Li, J. Liu, L. Zhang, W. Ye, X. Wu, Bioorg. Med. Chem. Lett. 19 (2009) 6966–6969.
[22] Y. Zheng, F. Zhou, X. Wu, X. Wen, Y. Li, B. Yan, J. Zhang, G. Hao, W. Ye, G. Wang, J. Ethnopharmacol. 128 (2010) 615–622.
[23] Z.N. Ji, W.C. Ye, G.G. Liu, W.L.W. Hsiao, Life Sci. 72 (2002) 1–9.
[24] Y. Bi, J. Wu, X. Wu, W. Ye, S. Yuan, L. Zhang, Bioorg. Med. Chem. Lett. 17 (2007) 1475–1478.
[25] H.J. Jeong, H.B. Chai, S.Y. Park, Bioorg. Med. Chem. Lett. 9 (1999) 1201–1204.
[26] J.Y. Kim, H.M. Koo, D.S.H.L. Kim, Bioorg. Med. Chem. Lett. 11 (2001) 2405–2408.